Researches on the Intestinal Protozoa of Frogs and Toads.

By

C. Clifford Dobell.

Fellow of Trinity College, Cambridge; Balfour Student in the University.

With 1 Text-figure (page 215) and Plates 2–5.

Contents.

Introduction.

The observations recorded in the following pages are the results of an attempt to discover the life-histories of the protists which inhabit the gut of the common frog and toad. My original intention was the investigation of the life-history of Trichomonas. But so great is the number of organisms which live in company with this form that I very soon perceived that it would be almost impossible to confine my investigations to a single species. Every organic particle in the alimentary canal had to be tested regarding its possible relationship to the organism which, in particular, I was examining. For example, I not infrequently found a number of cysts occurring side by side with Trichomonas in the frog's gut. To connect the two, without further evidence, would be ridiculous. I therefore had to discover to what organism the cysts belonged. And thus, time after time, I found myself driven to determine, to the best of my ability, the life-histories of all the protists which I encountered. The number of these is considerable. Therefore there were many difficulties in the way of success, and therefore, also, my work remains still far from finished.

My attention from the first has been chiefly directed towards the smaller protists, as these were relatively less known. There is but one of the larger forms, however—Opalina—which has really been carefully studied.

A part of my work has already been published—namely, that dealing with the little flagellate which I have called Copromonas subtilis (**10**); that dealing very briefly with a very small portion of the bacteria-like organisms (**11**); a preliminary notice of the organisms discussed in the present paper (**12**); a description of a portion of the life-history of the yeasts (**13**). I have also published some observations on a peculiar process of degeneration in Opalina (**9**).

I will preface my own observations with a brief summary of the work which has previously been done by others.

Historic.

I wish to give here only a general—and very brief—synopsis of the work which has been done upon the protists in the gut of the frog. I shall have to consider the various organisms in greater detail later, as I come to them. The subject is one of some interest, however, for it engaged the attention of some of the earliest microscopists.

Probably the first man to discover the existence of Protozoa in the intestine of the frog was van Leeuwenhoek, who, in 1683 ('Omnia Opera'), described and figured "animalcula in stercore Ranarum." These "animalcules" are generally supposed to have been Opalina intestinalis Ehrbg. Later, Leeuwenhoek carried his researches further, and was able—in 1702—to recognise three different protozoan "animalcula in the excrements of frogs." The species were, in all probability, Nyctotherus cordiformis Ehrbg., Opalina intestinalis Ehrbg., and another organism which was probably Trichomonas or Trichomastix—"Bodo ranarum" according to Ehrenberg.

For more than a century subsequently the subject received only brief and occasional notice. But I may mention during this period the names of Bloch (1782) and Göze (1782) who both devoted themselves—more or less successfully—to the study of these "intestinal worms" (Opalina, etc.). It was not until 1838 that any considerable advance was made. In this year—a landmark in the history of protistology—appeared the great work of Ehrenberg (**16**). Here we find that the author was able to distinguish no less than eight different species of protists. I give these below, with their probable synonyms in use at the present day:

1. Bodo ranarum Ehrenberg .= Trichomonas or Trichomastix.
2. Bodo intestinalis Ehrenberg .= Octomitus.
3. Bursaria ranarum Ehrenberg .= Opalina ranarum.
4. Bursaria intestinalis Ehrenberg. . . .= Opalina intestinalis.

5. Bursaria (?) cordiformis Ehrenberg. . . .= Nyctotherus cordiformis.
6. Bursaria nucleus Ehrenberg } = Balantidium entozoon.
7. Bursaria entozoon Ehrenberg }
8. Vibrio bacillus O. F. M. .= ?

Though necessarily imperfect, and in many cases highly fantastic, the descriptions of Ehrenberg remain in many ways a model of accurate and careful observation.

From the time of Ehrenberg down to the present day this little group of protists has received—with one exception—but scant attention. The exception is Opalina, of whose life-story, owing to the admirable researches of Zeller, Neresheimer (**40**),[1] Metcalf, and others, we now have a fairly perfect knowledge. As for the remainder, so little of the life-history has been discovered hitherto—the observations on them being mainly published in the form of short notes—that I will not discuss them further here.

Material and Methods.

The methods employed in the following researches have been, for the most part, the same as those which I have already described in a previous paper (**10**), to which the reader is referred. I will here add only a few remarks regarding one or two special points.

The frogs and toads have all been obtained either in Cambridge or in Munich. I have worked upon Rana temporaria L., R. esculenta L., and Bufo vulgaris L. As I have carried out the researches in two different laboratories the optical apparatus employed has been somewhat varied. But that has made no difference of any importance. The objectives, etc., employed will be found in the explanation of the figures, likewise the technique for fixing and staining. I may add, however, that the best fixation has always been obtained with Schaudinn's sublimate-alcohol, and the best staining with Heidenhain's iron-alum hæmatoxylin or Dela-

[1] A complete list of the literature on Opalina will be found appended to the work of this investigator.

field's hæmatoxylin. But I have used all the ordinary fixing fluids and stains. The greatest importance has always been attached to observations on the living animal.

As the organisms described naturally live in an anaërobic condition they are most suitably examined under tightly waxed-down cover-slips, and not in hanging-drop preparations.

It is almost impossible to obtain the contents of the frog's alimentary canal when required whilst the animal remains alive. Therefore I have had to resort to various means for obtaining the necessary material. I have frequently used the following method: A frog is taken and its brain (but not its spinal cord) pithed. When it has recovered from shock it is, of course, still quite lively, and will live for a long time. I pin the animal down in a dissecting dish, and by making an incision into the abdomen remove the contents of the large intestine by operating directly on it. If the frog be kept cool it will live for many days, thus enabling one to go on removing the gut contents at any required intervals of time. I have found the most suitable method of extracting the contents of the large intestine is to make a small cut into the small intestine at its juncture with the large. The contents of the large intestine can then be removed through the hole, and when sufficient has been extracted a piece of cotton can be tied immediately below the incision so as to close the large intestine once more. The frogs must be kept damp by covering them with wet cloths.

So many different organisms are to be found in the intestine of the frog[1] and toad that it will not be out of place to refer to these briefly at this point. In addition to the animals described in detail in subsequent pages, there are the following:

Among Protozoa we find Opalina ranarum Purk. et Val., Nyctotherus cordiformis Ehrenberg, Balantidium entozoon Ehrenberg, Balantidium duodeni Stein, and Balantidium elongatum Stein.[2] Copromonas is occa-

[1] Rana temporaria L. has been especially studied.

[2] First recorded by Dale (6).

sionally present. Of Bacteria there is an immense number of species, for the most part undetermined (cf. **11**), belonging to the genera Bacillus, Micrococcus, Spirillum, Sarcina, etc. Several species of yeast (cf. **13**) are also commonly to be found. And there are several different other fungi, the most remarkable of which is Basidiobolus ranarum Eidam. The cysts of this organism are common, and might be mistaken for those of Chlamydophrys or Copromonas, though they are usually a good deal larger. Other developmental stages of this very interesting fungus are also quite often encountered, as the cysts germinate in the fæces. Then the metazoan parasites must be mentioned. These are worms of different sorts—trematodes (Distomum, etc.), nematodes (Strongylus, Oxysoma, etc.), and an occasional cestode (Tænia dispar). The eggs of these forms—especially those of nematodes—are also usually to be found in abundance. The other organic particles which one encounters are chiefly degenerating epithelium cells and blood-corpuscles. Then, in addition, there are all the thousand and one undigested animal remains of the host's diet—remains of insects, bits of chitin, setæ of earthworms, fat droplets, etc.—together with shells of diatoms and desmids. I have also found the unopened and apparently intact spores of Monocystis[1] (from earthworms—several species) and Adelea ovata (from centipedes). Very many inorganic particles—e.g. various crystals, sand grains, etc.—are, of course, also present in greater or less numbers.

I will now proceed to the detailed description of the organisms whose life histories have especially engaged my attention.

A. Flagellata.

(1) The Trichomonads.

It has hitherto been universally supposed that but one trichomonad occurs in frogs, namely Trichomonas

[1] First noticed, I believe, by Lieberkühn (1854).

batrachorum Perty. There are, however, in reality two, a Trichomonas and a Trichomastix. I will begin with the latter.

(a) Trichomastix batrachorum Dobell.

I have already described this organism in my preliminary note (12). It differs structurally but little from other species, and is very common. It may occur alone, but is more commonly found in company with Trichomonas.

Structure.—In all essential points this animal's structure is identical with that of Trichomastix serpentis, which I have elsewhere described (56).

The general external form is usually ovate or pyriform, but subject to a certain amount of modification (see Pl. 2, figs. 1–3). The nucleus lies at the anterior end of the body, and is ovoid and composed of chromatin granules of irregular size and shape. A nuclear membrane is usually seen. Lying in front of the nucleus and generally in close apposition is a minute granule which stains with chromatin stains very intensely. This granule is often seen to be really double (cf. figs. 1, 3), and it serves as the point of origin of the four flagella. For reasons which will be apparent later I shall call this little diplosomic structure the blepharoplast.[1]

Of the flagella, three are directed forwards whilst the fourth is turned backwards (cf. fig. 3, etc.).

The flagella are not the only organellæ which find an attachment to the blepharoplast. A flexible rod-like organ is also firmly fixed to it, and runs backwards to end in the caudal process of the animal. This organ is one of the most characteristic features of the trichomonads, and although it has often been observed in Trichomonas, its significance has not always been properly understood. Its real function is undoubtedly skeletal. It serves as a fixed point for the anchorage of the flagella. Since the structure is one which

[1] The name was first used for trichomonads by Laveran and Mesnil (65).

occurs in more than one flagellate which I shall have to describe, and since no convenient name has yet been given to it, I propose to call it the axostyle[1]—a name which I think suitably describes it.

As in T. serpentis, the axostyle goes either through or over the nucleus to reach the blepharoplast. There can be little doubt that blepharoplast and axostyle are really united. That this is so is seen especially clearly in some cases where the end of the axostyle is bent (cf. fig. 2). I mention this because it has not been clearly made out by most investigators, e.g. by Prowazek in T. lacertæ.[2] The thickness of the axostyle is very variable. Two distinct types of organism can be thereby distinguished—a type with a very slender axostyle (fig. 3), and a type with a very thick one (fig. 2). Intermediate forms occur, but most of the animals can be classified under one type or the other. In the forms with a slender axostyle, one very frequently finds a few very intensely staining granules immediately above the point where it enters the caudal process (fig. 3). Their meaning is obscure.

A well-marked cytostome is usually to be seen (fig. 1).

The largest forms reach a length of 20 μ, from extreme anterior end to tip of axostyle. Very minute forms, about 6 μ long, are also found occasionally, but the average length is about 15 μ.

Although there is no visible cuticle, the animal does not exhibit as a rule any irregularity of contour. Only when it degenerates does it become amœboid. The cytoplasm is generally filled with food bodies.

The movements resemble those of Trichomonas, which have been often enough observed.

I have found the creature in Rana temporaria, but never

[1] The terms used by other writers are not few, and are mostly descriptions rather than names—e. g. "axial rod," "pointed organ," "baguette squelettique," "baguette interne," "style hyalin," "côte," "Achsenstab," "Rückenleiste," "Kiel," "Rippe," "costa," "bastoncello assile," etc.

[2] I have myself seen the attachment very clearly in this form on several occasions.

in R. esculenta or Bufo. It is less frequently present than Trichomonas.

Division.—Stages in division are very difficult to find. I had examined a very large number of living animals and had made over two hundred moist film preparations before I hit upon a single stage. When present they usually occur together. By making a large number of preparations I have been able to find practically every phase of division, though my observations on the living organisms have been fragmentary. I have not succeeded in following out the entire process from beginning to end in one and the same animal.

Division is longitudinal and takes place as follows (see Pl. 2, figs. 4—12) : The first thing to be seen is that the axostyle and nuclear membrane vanish, being apparently absorbed, so that a form like that shown in fig. 4 is produced. The chromatin lies freely in the neighbourhood of the blepharoplast in the form of small granules of varying sizes. Even at this stage (fig. 4) it can usually be seen that the blepharoplast itself is becoming elongated, assuming a dumbbell shape. It then becomes drawn out to such an extent that it takes on the appearance of a little rod. Two flagella remain at either end of this rodlet[1] (fig. 5). It appears to me that of the two granules which normally make up the blepharoplast one bears the posterior flagellum and the other bears the three anterior (cf. fig. 4). But during the division of the blepharoplast to form the rod one anterior flagellum in some way migrates over to the posterior flagellum, so that two flagella come to lie at either end of the rod (fig. 5). Next, the ends of the rodlet show an enlargement, so that the whole of the structure derived from the blepharoplast assumes the appearance of a very attenuate dumbbell (fig. 6). At the same time the chromatin granules, which were previously lying in a small indefinite heap, arrange themselves in the

[1] The origin of the flagella is not always made out with ease. For, owing to the way in which they get curled up, superimposed and entangled, they present appearances which at first sight are frequently very deceptive.

form of a spindle round the rodlet (fig. 6). I have never been able to make out achromatic spindle fibres at this stage (cf. Trichomonas, p. 217, and fig. 21).

At this stage, or perhaps earlier, the new flagella begin to make their appearance. They grow out from the thickened ends of the rodlet—which, from their subsequent development, I shall now call the daughter blepharoplasts—as four (i. e. two from each blepharoplast) small peg-like structures, which are easily recognised in Heidenhain preparations by their greater thickness and more intense staining. They do not always appear simultaneously (see figs. 7, 8, etc.). One now notices that the chromatin masses itself together into a few large, irregular, very strongly-staining lumps, which lie near the centre of the rodlet (fig. 7). The number of these masses varies, and they are usually difficult to count with accuracy. About six are present. They cannot justly be called chromosomes. It seems that the organism remains in this condition for some time, for it is the stage which is by far the most frequently encountered in stained preparations.

In a little while the chromatin heap becomes divided in two and each half travels along the rod, uniting the daughter-blepharoplasts, to take up a position by them (figs. 8, 9). The arrangement of the rod, blepharoplasts, chromatin masses and young flagellar outgrowths is particularly well seen in the specimen shown in fig. 9.

When it has reached the region of the blepharoplast each chromatin mass fragments and constitutes a new nucleus (fig. 10). During this process the rod becomes thicker and begins to stain less intensely (fig. 10). Hand in hand with the nuclear changes have gone changes also in the configuration of the cytoplasm. Whilst this was originally of a somewhat oval contour (figs. 4, 5), it passed through a stage of being roughly triangular (figs. 8, 9, etc.) to the present condition, which is more or less reniform in outline (fig. 10).

For a long time I was unable to find any further stage than this in my permanent preparations, although I searched long and carefully. The reason for this I then discovered from

observing the living animal. After this stage the animal completes its division with great suddenness. After remaining for some time in the state shown in fig. 10 a kind of constriction appears very suddenly in the middle (fig. 11). The constriction deepens all of a sudden, and then almost disappears again, appearing as though an unseen string were suddenly tightened and then loosened around the animal. This welling in and out lasts for several seconds, being repeated some half-dozen times, and then in a flash the creature is snapped in two by the constriction being completed, and two little daughter monads are left facing in opposite directions (fig. 12). For several seconds they remain thus, moving their flagella but feebly. Then they become more active by degrees and swim away from one another. It is seen that each monad possesses all the organellæ of the adult, and it is also perfectly plain that the rod which united the daughter blepharoplasts has, by dividing transversely, furnished each daughter monad with its axostyle. The axostyle is thus re-formed by the blepharoplast at each division. I will discuss the interesting points connected with this later (see p. 225).

The behaviour of the cytostome is not easy to make out during division. Very often, however, it can be quite clearly seen that the cytostome passes over into one of the daughter individuals (cf. fig. 10), so that the other individual must generate a new mouth. This is in agreement with Prowazek's observations on T. lacertæ (**73**).

Encystment.—After continuing to divide for an unknown length of time Trichomastix batrachorum is able to encyst. For a long time I was quite unable to find any trace of encysting in this animal. Even now I have not the remotest idea what causes encystment. In the ordinary course of events the animals, whether liberated in the fæces or removed by operation from the host, die sooner or later. And this happens no matter how they are treated—whether allowed to dry, whether placed in water, whether kept moistened in the fæces. All experiments to determine the cause of cyst-

formation have been negative. Neither change of temperature nor nutrition of the host appears to have the slightest influence. When I had almost despaired of ever finding the cysts I suddenly came upon them—in apparently quite ordinary frogs. It is curious—though perhaps a mere coincidence—that the cysts were all found in the months of November, December and January, before, and in part contemporary with, the period of cyst-formation in Opalina. When the cysts are present they are usually found in fairly large numbers, for many animals encyst at the same time.

Before encysting the animal undergoes considerable changes as regards its nuclear structure. Instead of the chromatin remaining distributed in the form of fine granules throughout, it begins to concentrate in the centre and in the nuclear membrane. The result is the formation of a nucleus with a sharp chromatic outline and a very distinct karyosome (fig. 13). A delicate thread is usually to be made out running in a longitudinal direction and uniting the karyosome with the membrane above and below (cf. fig. 13).

When the animal has reached this stage it begins to round itself off and decrease in size, preparatory to secreting a cyst wall. This process takes a very long time, so that it is almost impossible to follow it out in one and the same animal. However, I have seen every stage in different animals so many times that there can be no doubt about what occurs. The first thing that happens is that the axostyle begins to disappear, gradually dwindling away from behind forwards. As the caudal process ceases to exist the animal is able to round itself off. It does so, coming slowly to rest. After a time the movements of the flagella get slower and slower, and finally cease. Then the flagella disappear. They seem to dissolve, but it is difficult to see what becomes of them. It is just possible that they are drawn into the body, as in Copromonas in division. The blepharoplast remains behind, lying upon the nucleus (fig. 14). A diminution in size takes place, so that the organism shrinks to an oval mass of protoplasm. In this stage it forms the cyst membrane (fig. 14).

At first this is soft, but later it becomes harder and thicker. The axostyle gradually goes, and during the time of its disappearance a little darkly-staining, triangular area is generally visible between its remains and the nucleus (fig. 14). The significance of this is not apparent.

In the end the axostyle completely vanishes. The nucleus becomes drawn out in its long axis to such an extent that it often comes to stretch almost from one end of the cyst to the other (fig. 15). The karyosome also shares in the process, becoming drawn out into a long strand, which remains united to the membrane at either end. Above the nucleus, and in contact with it, the karyosome can generally be seen as a minute diplosomic structure (fig. 15). This stage is the last, and the cysts must now be regarded as permanent structures, which probably serve for the dissemination of the parasite. Although I have had cysts under observation for weeks at a time they have never undergone any further change. This is not difficult to determine, because although very small their structure can be made out quite clearly—with proper illumination, etc.—in the living state.

The cysts vary in size from ca. 4μ–$7\mu \times$ ca. 4μ–6μ, but average dimensions are ca. $6{\cdot}5\mu \times$ ca. 5μ.

The reduction in size in course of encystment is probably brought about by loss of water. It seems likely that before the reduction begins an actual diminution in the amount of solids in the composition of the protoplasm takes place. On several occasions I found that the large animals which were about to encyst were extraordinarily hard to fix. Instead of fixing in the ordinary way they collapsed, leaving only a few shreds of protoplasm and nucleus behind. The smaller animals—those in later stages of encystment—were fixed quite well however.

I have many times endeavoured to cause the animals to leave their cysts again, by treating them with the digestive juices of the frog. But all attempts have failed—a fact which I attribute to the abnormal condition of the laboratory frog, more especially in winter, when the experiments were made.

(Cf. similar results obtained with the amœba and coccidia, p. 253, etc.)

According to Prowazek (**73**), the division of Trichomastix lacertæ differs from that which I have just described. It appears from his account that the axostyle is drawn up towards the nucleus and then rearranges itself at right angles to its original position—passing through a T-shaped phase in doing so. The connection of the axostyle to the blepharoplasts was not made out. When the rod is rearranged the nuclear chromatin travels in two masses to each of its ends. The axostyle thus appears to function as a kind of division centre. From my own observations on this organism I believe that its structure and method of dividing are identical with those just described in T. batrachorum. But unfortunately I have found only a very few stages in division, so that I may be wrong. Some of Prowazek's figures, however, also support my interpretation (cf. figs. 8, 10, Pl. 1 [**73**]).

The method of division which I have elsewhere described in T. serpentis (**56**) also differs considerably from that of T. batrachorum. As my observations were made chiefly on living organisms, it is possible that I misinterpreted what I saw. Nevertheless I was able to watch division many times with great clearness, and believe the figures and description I have given are substantially correct for the living animal. The presence of a filament connecting the blepharoplasts after division may, however, have escaped my notice.

Prowazek (**73**) has described an autogamy in the cysts of T. lacertæ, but I have never seen anything at all like it in T. batrachorum. The cysts of the former species seem to be totally different in every way.

(b) Trichomonas batrachorum Perty.

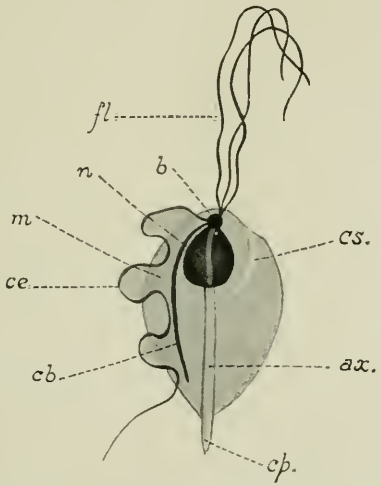
Syn.: [? Bodo ranarum Ehrenberg, 1838].
Monocercomonas batrachorum Grassi, 1879.

Cimænomonas batrachorum Grassi, 1882.[1]

Trichomonas batrachorum (Perty) Stein, 1878; S. Kent, 1880; Bütschli, 1884; Blochmann, 1884; Doflein, 1901, etc.

This animal was first recognisably described and named by Perty in 1852. I retain Ehrenberg's emended spelling of

TEXT-FIG.



Trichomonas batrachorum—diagrammatic. *n.* nucleus; *b.* blepharoplast; *fl.* flagella; *ax.* axostyle; *cp.* its caudal process; *m.* undulating membrane; *ce.* chromatic edge of same; *cb.* its chromatic basis; *cs.* cytostome.

the generic name introduced by Donné (Tricomonas, 1837, for T. vaginalis).

The occurrence of the parasite differs somewhat from that of Trichomastix. It is found not only in Rana tempor-

[1] Grassi here gives the following synonyms: "Cercomonas intestinalis ? Ehrbg.." "C. ranarum ? Ehrbg.," Bodo intestinalis ? Ehrbg., and Bodo ranarum ? Ehrbg. The first pair are really synonyms for the second pair. (Cercomonas = Dujardin 1841, used to replace Bodo Ehrbg. by Perty 1852.) Bodo intestinalis Ehrbg. is probably Octomitus (see further on under this heading).

aria but also in Rana esculenta and Bufo vulgaris.[1] It is quite probable that Trichomastix really also occurs in the last two, though I have never as yet encountered it there.

Structure.—Now that I have described the anatomy of Trichomastix it will be an easy matter to describe Trichomonas, for the two animals are alike in most respects. The only notable difference is that Trichomonas possesses an undulating membrane in place of the posterior flagellum of Trichomastix. The structure of the animal is shown in the accompanying text-figure. It will only be necessary to say something in addition about the undulating membrane (see also Pl. 2, fig. 16).

The undulating membrane resembles that of a trypanosome. It has a well-differentiated thickened border, which ends posteriorly in a free flagellum, as in Trypanosoma. This edge stains very intensely with iron hæmatoxylin, and to a less extent with other chromatin stains. In addition to this, however, there is also a rod-like chromatic basal structure, whose extent and degree of development vary a good deal. Sometimes it is represented merely by a few granules, arranged in a moniliform manner (as in the lower of the two membranes in fig. 20). Both the chromatic edge and the chromatic basis take their origin in the blepharoplast.

The rest of the animal's organisation is the same as that of Trichomastix (cf. figs. 1 and 16, Pl. 2).

The undulating membrane during life moves like that of a trypanosome. It will be superfluous to describe its movements.

It is surprising that so much uncertainty should have existed regarding the structure of this organella. Grassi (**21**) described it as a flagellum, but later (**22**) allowed that it might be a flagellum united to the body so as to form a kind of membrane. The discrepancy probably arose from his having observed both Trichomonas and Trichomastix. Stein

[1] And in Hyla arborea (Grassi).

had previously described it (**46**) as a "der Bauchseite angehörige Reihe von spitzzackigen, undulirenden Fortsätzen, welche gewöhnlich für Wimpern angesehen wurden." This investigator also saw the axostyle and nucleus. Seligo (**44**) inclined to Stein's interpretation of the structure, but Blochmann (**1**) and others clearly recognised its true nature.

The method of division, excepting as regards the membrane, is almost identical with that of Trichomastix (see figs. 17—24). I will therefore merely note the few points of difference.

The undulating membrane appears to be multiplied by splitting. In this the chromatic border takes part, but not the chromatic basis (figs. 17, 23). The latter never seems to split, but seems to be absorbed and reformed in each daughter-membrane. But it is not easy to see what happens to it exactly. The two membranes may become completely separated at quite an early stage (figs. 18, 20), or may remain attached posteriorly till quite late (fig. 23).

The blepharoplast and axostyle behave in exactly the same way as in Trichomastix (cf. figs. 5—10). The stage figured in fig. 21 is specially instructive. There is a very distinct spindle figure, much more clearly marked than anything I have ever found in Trichomastix. Moreover, the resemblance of the blepharoplasts to centrosomes is particularly striking (see discussion of this matter, p. 220 et. seq.). In the animal here figured the double nature of the blepharoplasts was also particularly clearly shown.

I have not found so many division stages in Trichomonas as in Trichomastix, but the likeness between them is so great that I have little doubt that they correspond almost identically.

Encystment takes place precisely as in Trichomastix. Before encysting the animal develops a karyosome in its nucleus (fig. 25). The axostyle, undulating membrane and flagella are then gradually lost as the cyst is formed (figs. 26, 27). Finally the nucleus becomes drawn out in the cyst, and it is almost an impossibility to tell whether any given

cyst belongs to Trichomonas or Trichomastix, so closely do they resemble one another (cf. figs. 28, 15). Only by seeing the living animals encyst can one make quite certain.

Here, as in Trichomastix, there are no signs of any sexual process, either heterogamic or autogamic (see p. 227).

The account I have given of the life-history of the trichomonads is so different from those of others that I must here say a few words regarding certain points.

First as regards division. This has been said to occur by several observers, but details of the process are most meagre. For instance, Seligo (**44**) merely mentions the fact that he saw longitudinal division in Trichomonas batrachorum. In Trichomonas lacertæ Prowazek (**73**) believed that longitudinal division resembling that of Trichomastix lacertæ took place, but he was unable to find all the stages and his figure is unconvincing. He further described a multiple division, which, from what I have seen in Trichomastix serpentis (**56**), I believe to have been really a degeneration phenomenon.

Kunstler (**63**) stated that Trichomonas intestinalis (from the guinea-pig) divided longitudinally, and remained active during the process. But he gave no accurate details.

A description of the division of Trichomonas intestinalis from the mouse has recently been published by Wenyon (**87**). According to him, "there is a division of nucleus, blepharoplast, and of the peculiar pointed organ which projects from the posterior end of the animal. The undulating membrane and its support with the flagellæ (sic) appear to be new formations." Later he states that the "pointed organ"—i. e. the axostyle—"divides by longitudinal division and is the last part of the animal to divide." If Wenyon's description be correct, then the trichomonads of the mouse divide in a manner which is totally different from that of the forms which I have investigated. It appears to me probable, however, that Wenyon is mistaken, and that the appearances he has seen and

figured have been wrongly interpreted. His figures, 13, 15, and 21 (Pl. 11) are really drawn from dividing animals, I believe. They correspond closely with what I have myself seen. But the remaining figures of "stages in division" are, I think, nothing more than degenerate or fused monads. I have seen many similar appearances and feel convinced that they have nothing at all to do with division. It is remarkable also that no figure is given in which a splitting of the axostyle is shown. On the contrary, figs. 15 and 21 show a single rod extending from nucleus to nucleus—an appearance scarcely explicable on the assumption that the rod is formed from the split halves of the former axial organ.

In the second place it is to be noted that the cysts which I have found are quite different from those described in allied organisms. For the most part the accounts given (Kunstler, Perroncito, etc.) are too indefinite to allow of comparison being made, but in one case at least (Prowazek [73]) there exists a full account of encystment, and it differs widely from what I have seen. But it is fruitless to discuss the matter further at present.

I have never found any signs of the formation of those curious rounded-off, half-encysted forms, which, according to Wenyon, occur in Trichomonas from the mouse, and which probably bring about infection. I do not believe any such condition occurs in the trichomonads from frogs and toads.

Finally, it is necessary to say something about the problem of hosts and species. All I wish to say is that the trichomonads I have observed appear to me to be sufficiently well marked to be kept specifically distinct for the present. As is well known, a Trichomonas intestinalis has been described from many different hosts. Whether there is really one species, or more than one, our present state of knowledge does not permit us to decide. Similarly, in spite of their great resemblance, I believe Trichomonas and Trichomastix are sufficiently well distinguished from one another to be placed conveniently in different genera, without entering

into the endless discussion of "What is a genus?" or "What is a species?" It seems to me profitless to argue the matter further at the present time.

(c) Discussion of some Special Points in the Morphology and Life-history of the Trichomonads.

I.

The Blepharoplast.—I wish here to say something about the minute chromatic[1] body—the blepharoplast—which lies at the base of the flagella, and whose remarkable rôle in division I have already shown.

The name "blepharoplast" was introduced by Webber (**86**) for the small body, which lies beside the nucleus and gives rise to the cilia in the antherozooids of plants (cycads, ferns, etc.). It was previously described by Belajeff, to whose beautiful work (**48, 49, 50**) we owe much of our knowledge of its nature. Additional facts have been givan by Ikeno (**60**), Shaw (**80**) and others. Although the earlier work was inconclusive, it seems practically certain, from the more recent studies—especially of Shaw and Belajeff—that the blepharoplast of the spermatozooid is really the centrosome or its derivative.

Amongst animals an exactly comparable condition—as I believe—is found. Here the axial filament of the spermatozoon tail arises, at least in many cases, from the centrosome—just as the cilia of the spermatozooid arise from the blepharoplast. This was first described by Moore (**69**), and has since been confirmed by a host of other workers (Hermann, Lenhossék, Meves, and many more).

Latterly the term "blepharoplast" came to be used to designate the chromatin body which lies at the base of the

[1] It can be stained not only by the iron-hæmatoxylin method, but also with Delafield's hæmatoxylin and borax carmine (not always satisfactorily with the last).

flagellar apparatus in trypanosomes—without, however, its strict homology with the organ in plant spermatozooids being insisted upon. Many different opinions have existed regarding the real nature of this body. Rabinowitsch and Kempner (**75**) regarded it as a "nucleolus," though why it is difficult to see. Wasielewsky and Senn (**85**) believed it to be merely a cytoplasmic thickening—a structure independent of the nucleus. Laveran and Mesnil (**65**) considered that the blepharoplast should be regarded as a kind of centrosome—a view which they had already advocated in 1900 ('CR. Soc. Biol.'). The view was based upon analogy with the structure of sperms and some flagellates, for no evidence of the blepharoplast functioning as a division centre had been brought forward. But it was a suggestive working hypothesis. Bradford and Plimmer (**52**) called the blepharoplast a "micronucleus," because they believed it played a part in the "conjugation" which they observed. This comparison with the organella of Infusoria probably rested upon an incorrect interpretation of the phenomena observed.

The whole matter was apparently cleared up by Schaudinn (**79**) in his study of the life-cycle of Hæmoproteus (Trypanosoma) noctuæ. From this famous investigation it appeared that the trypanosome blepharoplast should really be regarded as a nucleus specially differentiated to subserve the locomotory functions of the cell—a kinetonucleus, which played its part in conjugation, etc., just like any other nucleus. Far from being itself a centrosome, Schaudinn showed that it actually contained a division centre, just like that of the trophonucleus.

Starting out from Schaudinn's discoveries, Gross (**59**) made a very suggestive comparison between a sperm and a trypanosome, but in a converse manner to that which had been made by Laveran and Mesnil: that is to say, he suggested that the end-knob (centrosome) of the sperm might be regarded as a kinetonucleus, the sperm thus being binucleate like a trypanosome.

More recently a very careful investigation of the morpho-

logy of trypanosomes (T. gambiense, etc.) has been made by Salvin-Moore and Breinl (**70**). Their results are worthy of special attention, because their methods were greatly superior to those used by the majority of trypanosome describers. So convinced are these two investigators of the centrosome nature of the blepharoplast that they call it throughout "the extra-nuclear controsome." They give excellent figures of its origin from the "centrosome," which originally lies in the middle of the synkaryon. I must point out, however, that although the blepharoplast here appears to be the sister of the "intra-nuclear centrosome"—which seems to act as a division centre in the "amitosis" these authors describe—there is, nevertheless, no proof that it possesses the most characteristic powers of a centrosome, that is, in bringing about nuclear division.

The first observers to describe the trypanosome blepharoplast as playing the part of a division centre are França and Athias (**57**), who have lately figured some remarkable stages in T. rotatorium. They describe irregular, amœboid stages which undergo "segmentation," during which the flagellum is lost, and the blepharoplast appears to divide and function as a centrosome during the nuclear divisions. Although this fits in so well with the views which I hold, I must confess that these forms, both from the description of their origin and from the figures, seem to me to be abnormal or degenerate.

Hartmann and Prowazek (**25**) have sought to bring the blepharoplast of Trichomonas and Trichomastix into line with their expanded version of Schaudinn's "Doppelkernigkeit" hypothesis. They base their views upon Prowazek's description (**73**) of the forms from the lizard. The karyosome is regarded by them as the kinetonucleus, boxed up in the trophonucleus. Hence they say that "the basal bodies [1] at the root of the flagella can be interpreted as daughter-centrioles of the karyosome-nucleus, and hence correspond with the derivatives of the centrosome in the tail

[1] What I call the blepharoplast.

filaments of spermatozoa." My own interpretation, that the trichomonad blepharoplast and the end-knob (not in any way the karyosome, however) are homologous, seems to me to fit in with the facts much more satisfactorily.

And this brings me to the point to which all the foregoing remarks are converging. In short, I believe that the divers structures with which we have been dealing—blepharoplast of fern, trypanosome and trichomonad, end-knob of sperm, and hence also centrosome—are all strictly homologous structures. That they are identical one cannot, of course, say. For the trypanosome blepharoplast is not, except in a very wide sense, a cytocentre. But functionally they are identical; in each they give rise to the locomotor organs—tail filament, undulating membrane or flagella, cilia—of the cell to which they belong. And from their behaviour one would suppose that they not only give rise to these organs, but also remain to preside over their functioning after their formation.

I do not wish to enter into a full discussion of this difficult matter here, so I will content myself with a very few further remarks. To do justice to the subject one would have to review a greater mass of literature than there is room for in this paper—all the work, that is, dealing with the so-called Lenhossék-Henneguy hypothesis.

When I mention the end-knob of the sperm and the blepharoplast of a fern or trypanosome as homologous, I do not mean to imply that similar organs in other organisms are not to be included in the same category. Indeed, I believe there are many other quite similar arrangements. It will suffice to recall the condition described by Ischikawa (61) in the spores of Noctiluca. The centrosome is here seen to lie at the base of the flagellum, just like a blepharoplast. It is difficult to believe that it could be other than homologous.

Further, the conditions described by Schaudinn in Hæmoproteus noctuæ are not necessarily antagonistic to this hypothesis. Assuming that these unconfirmed observations of Schaudinn are correct, it does not follow that they apply

equally to ordinary trypanosomes. Indeed, the careful work of Salvin-Moore and Breinl on a true Trypanosoma show that quite a different arrangement exists here. The blepharoplast in T. gambiense does not appear to be a nucleus containing a division centre like that in H. noctuæ. It is quite possible that in forms like H. noctuæ the "blepharoplast" is really a specialised nucleus [1] which is in connection with the real blepharoplast. There are many cases known, moreover, in which one solitary nucleus gives rise to the flagella (cf. Plenge [72], etc.).

It is difficult to believe, from their structure, that the blepharoplast of Trypanosoma and that of Trichomonas (cf. fig. 16, Pl. 2) are not homologous. And obviously the blepharoplast of Trichomastix is homologous with that of Trichomonas (cf. figs. 1 and 16). But then, again, it appears more than probable that the blepharoplast of the trichomonads is homologous with a centrosome (cf. figs. 6, 21—especially the latter). When the case of sperms is considered in addition, the homology appears to me almost established.

I do not for a moment suppose that either of these structures —blepharoplast and centrosome—is derived necessarily from the other. They are, according to my view, merely homologous organs—both originally, in all probability, derived from the nucleus. Their morphological similarity depends upon their physiological identity. Their nuclear derivation is seen, in many cases, in their staining reactions.

These points seem to me to be very clearly brought out in

[1] Since writing the above remarks, I have been pleased to find that my view fits in exceedingly well with the observations of Minchin (68). I think his view really corresponds exactly with mine, namely, that we may have, in connection with the locomotory organs, a specialised nuclear apparatus which is really to be regarded as kinetonucleus + blepharoplast. Minchin agrees with Keysselitz and others in word only—not in idea. For him there are two structures at the base of the locomotor apparatus—a kinetonucleus and a blepharoplast of a centrosomic nature. Of course, it does not in the least follow that all trypanosomes are built on the same plan as those in tsetse-flies.

the study of the division of the trichomonads recorded in preceding pages. To conclude my remarks, I will now sum up the views which I have attempted to express as briefly as possible in the foregoing pages, in the following words: The blepharoplast of the antherozooid, the blepharoplast of the trypanosome and trichomonad, and the end-knob of the axial filament of the metazoan sperm are all homologous structures, whose function is to provide for the locomotory activities of the cell. They are further homologous with—in some cases (e. g. in sperms) directly derived from—the centrosome of the resting cell.

II.

The Axostyle.—This organ may suitably be considered here, as it is very closely connected with the blepharoplast.

Regarding the function of this organella in the adult individual there is some diversity of opinion. I believe that its real function is entirely skeletal. It is merely an axial support.

From Prowazek's work (**73**) it would appear to be a kind of division-centre in addition. But, as I have already said, I believe this conception rests upon an incorrect interpretation of the appearances observed. Nor can I agree with Wenyon (**87**) that the axostyle is an organ for attachment. One has only to observe the living animal to see that it is never used for this purpose.

What I am particularly concerned with here is the origin of the axostyle. As I have already shown, it is absorbed before division and reformed by the division of the blepharoplast. If the blepharoplast itself is the homologue of the centrosome, then the homology of the axostyle with the central spindle[1] at once suggests itself. The homology is

[1] I use the term in its original sense (Hermann, 'Arch. mikr. Anat.,' 1891), that is, for the spindle uniting the centrosomes, and around which the mantle-fibres of the achromatic spindle are arranged.

obvious, if we consider a stage, such as that shown in fig. 21, Pl. 2. The daughter blepharoplasts (centrosomes) lie at either end, united by the axostyle in its early stage of development. It clearly corresponds to a central spindle. Around it lie the mantle-fibres—never very strongly developed —and the chromatin, though never strictly divided into chromosomes, in the equatorial plate stage. Later stages in the development of the axostyle are fundamentally but stages of growth (e. g. fig. 9, 10, etc.).

It appears to me justifiable, therefore, to say that the axostyle is the homologue of the central spindle, each being a centrodesmus.

An almost similar conclusion has been arrived at by Hartmann and Prowazek (**25**), though in a different manner, and, as I believe, from incorrect premisses. The forms considered were the trichomonads from the lizard, following Prowazek's description. They say that the axostyle is formed by the "Caryosom des Amphinucleus," but I can find no foundation for this statement. And further, "Die vermutlich mit dem Centriol in Zusammenhang stehende Rippe (Achsenstab) ist eine Art von Centralspindel und geht in die Rippe des Tochtertieres über:" which is in complete agreement with what I have just inferred from my own observations.

Some other interesting comparisons may be adduced in favour of this view. Compare, for example, the origin of the tail filament—also a supporting structure—in spermatozoa,[1] by an exactly comparable centrodermosis, as described by Gross (**59**). And this also corresponds with the origin of the flagellum and membrane in trypanosomes and allied forms. And further, compare this with the origin of the flagella from the central spindle in the spores of Noctiluca as shown by Ischikawa (**61**).

In the remarkably complicated flagellate Joenia, Grassi found an organ which seems to be an axostyle. The division of this organism has been investigated by Grassi and Foà

[1] Of Pyrrhocoris. This method does not seem to obtain in most other sperms.

(**58**), and furnishes some interesting details. Before division the axostyle ("mestolo") is absorbed. Then a spindle ("fuso") of unknown origin makes its appearance beside the nucleus. It elongates enormously and comes to lie between the daughter nuclei; and subsequently a portion of it at least takes part in the formation of the axostyle in the two daughter individuals. It seems quite probable that a condition identical with that seen in the trichomonads really exists here, but that it was not fully made out owing to the great complexity of structure in Joenia. At all events, the comparison is very suggestive.

Another interesting comparison may be made between the axostyle and the axopodial rays of the Heliozoa. Camptonema furnishes an excellent example of the connection between axopodium and nucleus (Schaudinn [**77**]) and well illustrates a condition analogous to that of nucleus and skeleton in trichomonads.

III.

Conjugation.—From what has already been written regarding the life-cycle of the trichomonads from frogs, it will be apparent that I am quite unable to bring forward any evidence regarding their sexuality. At no time have I ever found the slightest indication of the existence of any form of conjugation.

It was stated by Schaudinn (**43**) that a conjugation (heterogamic) takes place in the Trichomonas intestinalis in man. This has never been properly confirmed. Shortly after, Prowazek (**73**) described an autogamy in Trichomastix and a heterogamy in Trichomonas intestinalis from the rat. Peculiar structures, said to be stages in conjugation (autogamy) in T. intestinalis in man, have since been described by Ucke (**84**) and Bohne and Prowazek (**51**). And it is to these very questionable bodies that I presume Prowazek refers (**74**) as autogamic stages. Personally I cannot agree with this interpretation of the structures. I have good reason for regarding them in a very different light.

And hence, for my own part, I regard the conjugation of Trichomonas and Trichomastix as still undemonstrated.

Negative evidence is, of course, always inconclusive. The fact that I have never found any conjugation in trichomonads after observing many, many thousands, proves nothing—save that the process, if it occurs, is very uncommon and difficult to find. But the difficulties I have met in the course of my researches have also shown me time after time the caution which is necessary in investigations of this sort. It is not justifiable from finding flagellates and cysts, or things like cysts, together in the gut contents, to connect the two without further evidence. As I have found only too often, it is necessary to study all the organisms which occur in the gut; and not only the organisms but also all cell-remains and other débris. Only by conscientious adherence to this slow and tedious method can satisfactory results be obtained. It is a pity that this elementary and obvious precaution has been so frequently neglected.

(2) The Octoflagellate (Octomitus dujardini nom. nov.).

Although this minute organism is the commonest of all the flagellates which are found in the large intestine[1] of frogs and toads, nevertheless it is the one which has given me the greatest trouble; and about its life-history I have been able to discover but little. On account of its very small size and very complicated structure it is not surprising to find that it has never been accurately described. None the less it has been named a great many times, with the result that the literature and the available facts relating to it are at present in a hopelessly chaotic condition.

I will therefore first endeavour to summarise briefly the

[1] Since writing this account of the parasite it has been pointed out to me by Prof. Minchin that Danilewsky ('Parasitologie Comparée du Sang II,' 1899) observed the organism in the blood and body-cavity of sickly frogs, etc. This must be regarded most certainly, I think, as a pathological condition.

history of the animal. I have found it impossible to name without an exhaustive inquiry into all the available literature bearing upon it.

Only one serious attempt, that of Foà, has been made recently to classify this animal correctly, and her solution of the matter cannot be regarded as correct. At the time when I published my preliminary account I was unable to enter fully into a discussion of the matter. I therefore named the organism Octomitus sp., and I will now give my reasons for having done so.

The first authentic record of flagellates in frogs, so far as I have been able to discover, is that of Ehrenberg, 1838.[1] Ehrenberg distinguished two different organisms: Bodo intestinalis, and Bodo ranarum. The former he states to be $\frac{1}{72}$ mm. long, occurring in the large intestine of frogs, the latter $\frac{1}{60}$ mm. long, and found in frogs and toads. His description and figures are naturally very incomplete on many points (e.g. the number of flagella), but it appears to me highly probable that B. intestinalis Ehrbg. is really the 8-flagellate parasite, and B. ranarum Ehrbg. is Trichomonas or Trichomastix. I think it is certain that neither really belongs to the genus Bodo as at present constituted.

In 1841 Dujardin established the genus Hexamita. He described three species: H. nodulosa and H. inflata, from stagnant water, and H. intestinalis. Unfortunately only H. nodulosa is figured. It shows six very distinct flagella. H. intestinalis is stated to occur in the intestine and peritoneal cavity "des Batraciens et des Tritons." There appears to me to be but little doubt that this was really the 8-flagellate parasite—only six of whose eight flagella Dujardin was able to count with the apparatus at his disposal.

Diesing in 1850 describes, though apparently without any justification, the Hexamita intestinalis of Dujardin under the new name of Bodo (Amphimonas) decipiens Diesing. He makes no original observations on the organism.

[1] But they were possibly first observed by Leeuwenhoek in 1702.

Burnett, 1851, mentions the presence of "Bodo (Ehr.)" in the frog, and records a few observations on the organisms. But I am quite unable to decide which of the flagellates in the frog he really saw.

Perty was the first, in 1852, to distinguish Trichomonas batrachorum from the other flagellates. But he also appears to have recognised a flagellate which he calls Cercomonas ranarum (Bodo sp. of Ehrbg.). Probably this was the 8-flagellate once more, under another name.

Leidy, 1856, recognised both Ehrenberg's forms of Bodo, retaining the latter's name, B. intestinalis, for the smaller form.

The next change of name was brought about by Diesing, 1865. He describes Hexamita intestinalis Duj. as Amphimonas intestinalis. This name cannot be retained. The genus Amphimonas was made by Dujardin in 1841, and included three free-living species, each possessing two or three flagella. There is no justification for Diesing changing Dujardin's own genera in this way.

Stein's great work on flagellates appeared in 1878, and in it he describes, with tolerably accurate figures, a parasite said to be common in frogs, under the name Hexamita intestinalis Dujard. Although Stein only figures six flagella, I think there can be no doubt that he really saw the 8-flagellate organism. The rest of his description is fairly good.

Bütschli, in the same year (1878), resumed the investigation of the free-living forms. He states that there are really eight flagella in these organisms and unites Dujardin's two species, Hexamita nodulosa and H. inflata, into one species, Hexamitus inflatus, thus modifying the original name. It must remain doubtful whether Butschli's 8-flagellate organisms were really the same as Dujardin's 6-flagellate animals.

Further complications were brought about by Grassi in 1879. He proposed the generic name Dicercomonas for two different parasitic flagellates. The genus was dis-

tinguished from his other genus Monocercomonas (Trichomonas of others) by the one character "a coda bifida." He divided the genus Dicercomonas into two sub-genera, Monomorphus and Dimorphus, of which the definition is, to say the least, scanty. Monomorphus is distinguished as "si presenta sotto una sol forma." The only species is Dicercomonas (Monomorphus) ranarum, with "Hexamita ranarum, Duj."[1] given as a synonym. The name Dimorphus was given to D. muris, and subsequently eliminated as Megastoma entericum (Grassi, 1881).

Saville Kent, 1880, re-described Hexamita intestinalis Duj. from his own observations. His account is in many ways inaccurate, and he persists in the statement that there are six flagella: "The exact number, character, and point of insertion may be readily substantiated . . . " I feel convinced that he really saw the 8-flagellate organism. He enumerates further both Ehrenberg's Bodo forms, but made no observations himself upon them. He suggests, however, that Monas intestinalis Dujardin is a synonym for Bodo intestinalis. This appears to me highly improbable.

Grassi re-described the organism under consideration in 1882. He was unable to determine the number of flagella, and apparently relinquished the name Monomorphus. For he adheres to the name Dicercomonas intestinalis Duj., giving Hexamita intestinalis Duj. as only synonym.

In the same year Kunstler (1882) described—though very briefly—a flagellated organism from tadpoles, which appears to me to have been probably our 8-flagellate organism. The flagella were not accurately investigated. Kunstler, in spite of his insufficient observations, introduced the new name Giardia agilis for this animal.

Bütschli, 1884, retains the genus Hexamitus[2] (Duj. emend. Büt.).

[1] A mistake for Hexamita intestinalis Duj.

[2] Giving Chætomonas Ehrbg. and Heteromita pusilla Perty as possible synonyms for Hexamitus. Neither of these appears to me to have anything to do with the form under consideration.

In 1885 Seligo again described a 6-flagellate parasite from various frogs, etc., employing the name Hexamitus intestinalis Duj. for it.

Grassi, 1888, maintained his former genus Dicercomonas, but gave a better definition. He, however, recognised only "quattro flagelli anteriori." He gave as synonyms Hexamita Duj. and Giardia Kunstler. For the free-living forms he proposed to replace the name Hexamita Duj. by the new name Dujardinia Grassi, which, if adopted, would thus abolish the name Hexamita entirely.

Klebs, 1892, retained Bütschli's nomenclature (Hexamitus intestinalis, Duj.), though recognising for the first time that this flagellate really possessed "stets sechs vordere und zwei hintere Geisseln, so dass die Gattung eigentlich Octomitus heissen müsste." And he adds, "Doch erscheint es passender, den alten eingenbürgerten Namen zu bewahren."

Senn, in 'Engler and Prantl,' 1900, also retains the name Hexamitus intestinalis Duj., and gives as synonyms for Hexamitus, Heteromita pusilla Perty, Amphimonas Diesing, and Dicercomonas Grassi—evidently copied from Bütschli. Four pairs of flagella are described.

Doflein, 1901, again attributes but six flagella to this animal, and retains Bütschli's name.

Stiles, 1902, made an attempt to arrive at a definite understanding regarding the nomenclature of this and other flagellates, but his work was entirely of a literary nature, and not based upon any further investigation of the organisms themselves.

Moroff, in 1903, was responsible for yet another change in the name of this parasite. He observed a similar organism in a fish, but stated (though his figures and description do not bear this out) that it was the same as that found in Amphibia, and there known as Hexamitus intestinalis Duj. He proposed to change the name, however, to Urophagus intestinalis (Duj.) Moroff,[1] because of the presence

[1] Wrongly giving Hexamitus intestinalis Dujardin, 1841, as synonym.

of eight flagella. How absolutely unwarranted such a change is will easily be seen when it is recollected that the genus Urophagus was founded by Klebs himself, the first to observe eight flagella in the parasitic form. And Klebs founded this genus to contain a single species, which differed from all the other 8-flagellate organisms in the fact that it ingested food at the hind end of the body—an act which Moroff never observed.

The first real attempt to describe the structure of this animal was made by Foà, 1904, who also made an attempt to assign the correct name to the organism. She says: "Grassi (1888) conferma la propria classificazione," and accordingly names the parasite Dicercomonas intestinalis (Duj.).

Now I must point out that this name adopted by Grassi and Foà is not available. The genus Dicercomonas was founded by Diesing in 1865,[1] and not by Grassi in 1879. Diesing's definition runs as follows: "Dicercomonas Diesing (monadis spec. Perty). Animalcula solitaria libera symmetrica. Corpus immutabile, ovale, hyalinum, caudiculis duabus retractilibus, nec ciliatum, nec loricatum. Os terminale. Flagellum unum pone os. Anus . . . Ocellus nullus. Partitio . . . Anodontarum parasita." He then enumerates a single species, Dicercomonas succisa Diesing (syn. Monas succisa Perty) found "in aqua cum Anodontis putrescentibus." It thus appears that Grassi's name must be relinquished, although it is just conceivable that Diesing really observed a similar organism, for Certes (1882) described Hexamita inflata Duj. as occurring in the oyster. However, we must take Diesing's definition as it stands—as that of a uniflagellate.

Finally, Kunstler and Gineste, in 1907, described another species of "Giardia," namely Giardia alata K. et G. from tadpoles of a frog. From their description this hardly seems to agree with my observations on the structure of the parasite under consideration. On the contrary, the new form appears more closely allied to Lamblia. However, it is just

[1] Not 1856, as given by Stiles.

possible that it is really the common form from the frog, and hence this must be considered as a conceivable synonym.

Now if we agree, as is usual, to take Hexamitus inflatus as the type species—a form with six flagella, and of free-living habit, as described by Dujardin—we are left without any generic name to bestow upon our parasitic form. Bodo Ehrenberg is unavailable; so also Amphimonas Dujardin and Dicercomonas Diesing. Cercomonas and Urophagus are quite distinct genera, and Giardia is too inadequately described to be adopted with certainty. That is why I proposed (**12**) to employ the name Octomitus, as originally suggested by Klebs. This name seems to me to be the most suitable for this and similar forms. But if we agree to call the animal by this name another difficulty at once presents itself. The genus Octomitus was created by Prowazek[1] in 1904 to include a single species, O. intestinalis from the rat. But this is the very name—Octomitus intestinalis Duj.—which our parasite would have to receive. Hence we arrive at another obstacle. Now it is quite probable that Prowazek's organism is only a form of the animal usually described as Hexamitus or Dicercomonas muris Grassi. Wenyon, however, thinks that there are probably two different species included under this title, in which case it would be best to let Prowazek's name stand.

I propose, therefore, to create the new specific name dujardini for the octoflagellate parasite of frogs and toads, whilst referring it to the genus Octomitus. This, I believe, will effectually surmount all difficulties, and will also take cognisance of the probable discoverer of the animal.

The genus Octomitus will, therefore, contain three[2] species of parasitic flagellates, namely:

1. Octomitus dujardini, in frogs and toads.

2. Octomitus muris Grassi, in rats and mice (the narrow form of "Hexamitus [Dicercomonas]" muris).

[1] Though written by him "Oktomitus," and without any indication that the name was being employed for the first time.

[2] The forms in tortoises, fish, oysters, etc., are too little known to warrant the giving of specific names to them at present.

3. Octomitus intestinalis Prowazek, also in rats (the broad form).

Hence I can now sum up the nomenclature, and will then proceed to a consideration of the organism itself. The name stands as follows:

OCTOMITUS DUJARDINI nom. nov.

Syn.: ? Bodo intestinalis Ehrenberg, 1838.
Hexamita intestinalis Dujardin, 1841.
? Bodo (Amphimonas) decipiens Diesing, 1850.
? Bodo (Ehrbg.) Burnett, 1851.
? Cercomonas ranarum Perty, 1852.
? Bodo intestinalis (Ehrbg.) Leidy, 1856.
Amphimonas intestinalis Diesing, 1865.
Hexamita intestinalis (Duj.) Stein, 1878.
Dicercomonas (Monomorphus) ranarum Grassi, 1879.
Hexamita ranarum (Duj.) Grassi, 1879.
Hexamita intestinalis (Duj.) Kent, 1880.
? Bodo intestinalis (Ehrbg.) Kent, 1880.
Dicercomonas intestinalis (Duj.)Grassi,1882.
? Giardia agilis Kunstler, 1882.
Hexamitus intestinalis (Duj.) Bütschli, 1884.
Hexamitus intestinalis (Duj.) Seligo, 1885.
Dicercomonas intestinalis (Duj.) Grassi, 1888.
Hexamitus intestinalis (Duj.) Klebs, 1892.
Hexamitus intestinalis (Duj.) Senn, 1900.
Hexamitus intestinalis (Duj.) Doflein, 1901.
Hexamita intestinalis (Duj.) Stiles, 1902.
Urophagus intestinalis (Duj.) Moroff, 1903.
Dicercomonas intestinalis (Duj.) Foà, 1904.
? Giardia alata Kunstler et Gineste, 1907.
Octomitus sp. Dobell, 1908.

Structure.—The general shape of Octomitus dujardini is fusiform or elongate oval (see Pl. 3, figs. 29, 31). An average adult individual measures about 10 μ in length. The nucleus and the organellæ connected with it present a consider-

able degree of complexity. The nucleus itself may be regarded as consisting of three pairs of structures. These all lie at the anterior end of the animal, and are arranged roughly in the shape of a horse-shoe. At the extreme anterior end are two minute granules of chromatin, lying side by side, connected with one another by a delicate filament of chromatin, or else in close apposition. Immediately behind this pair, and united to it, is another pair of chromatin granules. These are also connected with one another across the middle line. It will thus be seen that the two pairs of granules form the four corners of a minute square area, free from chromatin (cf. fig. 29). The main part of the nucleus consists of a large lobe of chromatin on either side, connected with, and extending backwards from, the posterior pair of chromatin granules.

Extending backwards from the posterior pair of granules are two delicate rod-like structures, which I believe to be homologous with the axostyle of trichomonads. I shall therefore employ the same name to describe them. Each axostyle terminates at the extreme posterior end of the animal in a minute chromatic granule. The eight flagella arise as follows: From the anterior end six, from the posterior two. The anterior take origin from the two pairs of chromatin granules, one pair of flagella arising from the anterior, and a single flagellum arising from the posterior on either side (cf. fig. 29). The posterior flagella, or, as I shall call them, the caudal flagella, arise from the chromatin granules at the posterior extremities of the axostyles. The length of the flagella is variable, but is frequently great. I have not unfrequently found individuals in which the caudal flagella had attained a length of over 30 μ, or more than three times that of the body. In consequence of their length and the minute dimensions of the animal there is often great difficulty in counting these appendages.

The axostyles are normally parallel, but they frequently get crossed, owing to the twisting movements of the animal (see fig. 30). This crossed condition, therefore, cannot be regarded as the normal condition, though the Octomitus in the rat

has been usually so described. Anyone who will take the trouble to watch an Octomitus continuously for several hours can convince himself of this. When the animal is moving quietly and has ceased to dart about, the axostyles invariably appear parallel with one another (see fig. 31). This crossing of the rods during active screw-like movements, moreover, negatives the suggestion of Prowazek that these structures, in O. intestinalis, are really not rods, but the sides of a tube seen in optical section. The axostyles have also been frequently interpreted as continuations of the caudal flagella (cf. Foà, etc.)

I have described the nuclear apparatus as it appears to me most usually to exist.[1] But there are other variations often met with. It is very commonly found that the two anterior pairs of granules are fused or superimposed, so that they cannot be made out clearly (cf. fig. 30). Many considerations have led me to believe, however, that the nuclear chromatin is really arranged in the three pairs of parts which I have described. A very striking confirmation is seen in a degenerate form, which is not uncommon in old cultures (see fig. 37, Pl. 3). In this the nucleus has degenerated and broken up, but into three pairs of granules. These forms have died and cast off their flagella. The nucleus has been resolved, I believe, into its component parts.

Very many other degenerate forms have been encountered. I will here mention only one more, which is very striking in appearance. In this (see fig. 36) the nucleus has fragmented, and the fragments have run along the axostyles, so that they present the appearance of strings of beads.

There is no cytostome and no contractile vacuole.

Octomitus dujardini occurs in Rana temporaria, R. esculenta, and Bufo vulgaris, and is equally common in all of them. It occurs also in newts.

[1] It is worth noting the extraordinary way in which all the parts of the nucleus and its connections are paired, thus giving rise to a very well-marked bilateral symmetry. It is interesting, too, to compare this form with other similar forms, e. g. Lamblia (cf. Metzner [66]).

I must here say a few words about some of the descriptions which have previously been given of this animal.

Stein (**46**) described it as having six flagella and a spherical "kernähnliches Körperchen" at the anterior end. He also described a terminal mouth and a contractile vacuole, and figured forms "mit zwei seitlichen Reihen undulierender Fortsätze am Vorderleibe." The axostyles are recognisable in some of his figures.

Saville Kent (**26**) says there is a "spherical, subcentral endoplast," and the body is "frequently with one or two longitudinal dorsal sulci" (? the axostyles). A contractile vacuole is said to be present, and is figured at the anterior end. Seligo (**44**) also found a bladder-like nucleus with a nucleolus lying near the middle of the body.

Apparently the axostyles were first recognised by Grassi (**23**), for he describes the organism as possessing a "scheletro interno (fatto da uno o due pezzi?)" The axostyles were indicated by Klebs also (**27**), when he described the body as being furnished "mit zwei schraubig verlaufenden seichten Längsfurchen, von denen je ein Rand stärker als Längskante vorspringt." No contractile vacuole was observed by him, and the nucleus was stated to be anteriorly situate.

The most accurate account yet given is that of Signa. Foà (**18**). She describes the anterior flagella as arising, three on either side, from a pair of "blepharoplasts," and interprets the main lateral lobes of the nucleus as "karyosomes." And she also saw a figured two longitudinal lines (the axostyles) running down the body. Her account is evidently based on careful observations.

The body so often described in the middle of the animal as the nucleus was probably a food mass. Such masses are often present, though how they get there I do not know, as there is no mouth. During degeneration, large vacuoles usually appear in the protoplasm, a large one often making its appearance immediately behind the nucleus. It is these vacuoles—which are not normally present—which have probably been taken for contractile vesicles. The frequently

seen bifid condition of the caudal extremity is also not a constant feature. It owes its formation to the rigidity of the skeletal rods and the mobility of the cytoplasm in which they are imbedded.

I have never seen anything corresponding with the undulating processes described by Stein.

So far I have described the structure of adult individuals only. But in addition to these there are usually to be found a certain number of small forms. Many of these are exceedingly minute—not reaching a greater length than 2–3 μ—and are of a simpler structure than the fully grown animals. Even in the smallest forms, however, when it is possible to make an accurate count of the flagella, there are always eight present. But some of the tiniest organisms appear to have only one axostyle (see fig. 32, Pl. 3). Stein has figured the young form with four flagella and one axostyle.

The shape of the smallest individuals is more rounded than that of adults. It is the nuclear apparatus, however, which shows the greatest differences. In the smallest forms (see fig. 32) the nucleus consists of a few loosely packed chromatin granules, and all the anterior flagella appear to be rooted in it. At other times the nucleus has a distinct karyosomic granule, and the flagella arise from minute basal granules on the periphery (see fig. 33). Later stages show a gradual transition to the bilobed nucleus of the adult (fig. 34), and many very small animals appear—as far as details can be made out—to be identical with adult individuals (fig. 35). The origin of these small forms is still unknown to me.

Movements.—When freshly removed from their host these animals display a remarkable degree of activity. They move at such a pace that it is quite impossible to make out anything of their structure as they dart across the field of the microscope—a mere dot of protoplasm surrounded by a blur of flagella. After a short time, however, they slow down, and one is able to watch their movements with ease.

In a slowly moving animal all the details of structure—save the most minute points in connection with the nuclear apparatus—can be made out, with patience, with almost as much certainty as in a stained specimen.

The body is characterised by extreme flexibility, which enables the animal to double and twist itself in all directions. Movement always occurs in a forward direction—that is, with the nuclear end in advance.

During progression it is only the anterior flagella which are lashed about. The caudal pair are usually trailed. Not uncommonly they become attached to some object, and thus serve to anchor the organism, which may then rotate about the fixed point. Saville Kent and others have already noticed this.

Multiplication.—In spite of having examined countless thousands of individuals, both alive and in fixed and stained preparations, I am still uncertain of the method of reproduction. I have many times found stained specimens which are identical with those described as division-stages by Foà and Wenyon in the Octomitus in rats. But from observations on the living animals I am now satisfied that these stages are merely degenerate and fused forms, which have nothing whatever to do with division. Bütschli states that "Theilung" occurs in Hexamitus inflatus, but beyond figuring an animal with two spherical nuclei and four caudal flagella he gives no details of the process. According to Prowazek, in Octomitus intestinalis "bei der Teilung scheint die Achsenröhre[1] ganz nach Art des Achsenstabes der Trichomonaden und -mastiginen zu funktionieren. Sie nimmt eine etwas spindelförmige Gestalt an, die vornehmlich durch eine Anschwellung des äusseren Belages hervorgerufen wird." Nothing further is said or pictured of the division.

I have on several occasions found stained specimens which appear to me to represent genuine stages in division. These are unfortunately extremely rare, and have all been at approxi-

[1] The axostyles were thus interpreted.

mately the same stage. It is therefore impossible to describe the whole of the process.

Two of these stages are figured in Pl. 3 (figs. 40, 41). According to my interpretation it appears probable that division is longitudinal and effected in the same way as in the trichomonads—allowing, of course, for the more complex structure. Before division the axostyles would therefore be absorbed, and with them the caudal flagella. A stage with only six flagella—all at the anterior end—would thus result. We do, indeed, find such organisms on rare occasions (see fig. 46), but I am inclined to think that they belong to a different species (see p. 245). However, they possibly belong here. The nucleus would subsequently divide, new flagella would make their appearance, three at either end, and we should expect to see two axostyles lying between the nuclei as they separate. This is the condition which I imagine is seen in figs. 40 and 41. Later, when the axostyles had elongated and the animal had been constricted into two, the caudal flagella would make their appearance, either by a new growth or by the drawing out of the axostyles at the point of severance. Both the organisms figured were very distinct, and in fig. 41 the suggestion of the outgrowth of new flagella, as in Trichomastix, is very strong. The bipartite nuclei are also very striking, and it seems difficult to regard these forms otherwise than as division stages. But as I have already indicated, the evidence of division is by no means conclusive. I give these few observations because I have completely failed to discover anything more, and because the descriptions of division in similar forms seem to me to be incorrect.

Encystment.—When the organisms are artificially removed from their host or liberated in the fæces they nearly always die. For a long time I was unable to discover the cysts or the method of dissemination in nature. On a few occasions, however, I have found the permanent cysts of Octomitus, though they have never occurred in anything but very small numbers. The cysts are small and usually

oval (fig. 38, Pl. 3), are slightly yellowish, and contain a single individual. The axostyles are not as a rule very distinctly seen, and there are no flagella present.

On a single occasion I have seen a cyst containing a monad which had become motile, having eight flagella, inside its cyst (fig. 39). After moving about actively, stretching the cyst in all directions, the monad subsequently escaped and swam away.

As in the case of the trichomonads, I have absolutely no idea what the influence is which causes the animals to encyst. Temperature, nutrition, drying, etc., appear to take no part in bringing about encystment.

Regarding sexual phenomena, I can merely repeat that I have never seen any conclusive evidence that conjugation takes place. Prowazek has stated that conjugation (heterogamy) occurs in "Hexamitus intestinalis" from Testudo græca, but I cannot regard it as proven. The conjugation is said to be similar to that of Trichomonas. It may be added that Wenyon's careful investigation of similar forms in the rat resulted in observations similar to mine—namely, the discovery of monozoic cysts without any trace of conjugation.

(3) Monocercomonas bufonis Dobell.

On two occasions I have encountered in the toad a quadriflagellate parasite, which differs considerably from Trichomastix. Although rare, the organism was present in great numbers in the infected animals. These, it may be noted, were both captured in the same place.

I have referred the animal to the genus Monocercomonas Grassi, because I believe the genus Tetramitus, to which similar organisms belong, ought to be reserved for free-living forms. And although the genus Monocercomonas is not very well defined,[1] it has already been used for

[1] The type-species is probably Monocercomonas melolonthæ, but Grassi's descriptions and figures are not easy to deal with. He has variously given Trichomonas (1879) and Trichomastix (1888) as synonyms for Monocercomonas.

parasitic flagellate forms with four equal anterior flagella. I think it best to retain this genus, therefore, for the parasitic quadriflagellates—reserving Tetramitus for free forms.

The general structure of the animals is shown in figs. 49 and 50, Pl. 3. Two different forms are here seen—a small, slender Crithidia-like form (fig. 49) and a larger and broader one (fig. 50). The size of the small forms is about $10\,\mu$–$12\,\mu \times 2\,\mu$. The larger forms reach dimensions up to $20\,\mu \times 7\,\mu$. All intermediate sizes occur.

One of the features which most markedly distinguish this organism from those already described is the presence of a very well-marked cuticle. This is best seen, perhaps, in Giemsa preparations, where it stains pink, in contrast with the blue cytoplasm.

The four flagella are equal in length, and are all directed anteriorly: that is to say, there is no "Schleppgeissel" as in Trichomastix.

The nucleus is a large, oval body, composed of loosely-packed chromatin granules. It is placed anteriorly. The origin of the flagella is immediately in front of the nucleus. Sometimes they appear to arise directly from it (fig. 49), whereas at other times they seem attached to a small granule lying above and independent of the nucleus (fig. 50). Several vacuoles are usually seen in Giemsa preparations, but these are not visible in the living animal. There is no cytosome or axostyle.

When alive the organism progresses by characteristic jerky movements, rather like a Bodo. They are exceedingly active when first removed from their host.

Owing to the paucity of material I have not been able to ascertain anything of the life-history. In cultures of the toad's fæces all the animals died without showing any signs of encysting. From the fact that I have several times observed —in stained preparations—animals with eight flagella, it appears probable that they divide longitudinally in the usual flagellate manner. But the nuclear division and subsequent stages I have not been able to find.

(4) Notes on other Flagellate Organisms.

In the course of my work I have come across several doubtful organisms, which I will briefly describe here. They have been found, for the most part, in fæces cultures. I believe that they are in no way related to the other forms described in this paper, but that their presence was due to accidental inoculation of the cultures. However, their possible connection with other forms is not excluded, and I will therefore describe them. They are all of them uncommon.

1. A minute uniflagellate monad (Pl. 3, fig. 47). Length 3 μ–6 μ. Sometimes shows a tendency to become amœboid. Stained specimens show a nucleus centrally situated, and consisting merely of a minute chromatin granule. Seen on several occasions in fæces of Rana temporaria and once in Bufo vulgaris.

2. Bodo sp. (Pl. 3, figs 42–45). Found on two occasions in fæces of Bufo.[1] Shows typical Bodo structure—two flagella, etc. (fig. 42). Length, up to 15 μ. Hinder end often becomes amœboid, forming hyaline pseudopodia (fig. 43). Nucleus central. Very tiny forms sometimes seen (? another species), not measuring more than 3 μ in length (fig. 44). On one occasion I found—in a preparation with free forms—a cyst which appeared to contain four Bodos and a residuum (fig. 45). As no flagella could be seen it is possible that the cyst belongs to some other animal. But it is interesting to record its presence, since Bodo may divide into four, after encysting, according to Prowazek. The length of the cyst was 21 μ. I was unable to break it, owing to the presence of much sand in the fæces preventing the coverslip from being pressed against the slide. Though watched for several hours no movements of any sort took place.

3. A triflagellate monad (fig. 48, Pl. 3). Found only once,

[1] I have found a very similar Bodo parasitic in the large intestine of the common newt. It is remarkable for the possession of a very large blepharoplast-like body (Geisselsäckchen) at the base of the flagella.

in small numbers, in fæces of Bufo. The three flagella are separated at their origin, and equal in length. Length about 6 μ. Movements sluggish.

4. An organism with six flagella. Several specimens found on different occasions in the fæces of Bufo vulgaris and Rana temporaria. Nucleus spherical, granular, anterior in position. Six equally long anteriorly directed flagella. No axostyles. Length about 10 μ (Pl. 3, fig. 46.) May possibly be a degenerate or developmental form of Octomitus (cf. p. 241), with which it was always found.

I may add that I have several times observed the Bodo described above undergo a process of degeneration which is remarkable for the formation of long, delicate, heliozoon-like pseudopodia. In this radiate condition the animal bears some resemblance to the multiciliate creature described in frogs by Grassi. According to Schuberg this is really a detached epithelium cell, but Grassi denies this. The name Grassia ranarum was given to it by Fisch. I regard its existence as highly doubtful.

B. Rhizopoda.

(1) Entamœba Ranarum Grassi.

Syn.: "Amöbe" Lieberkühn, 1854.
Amœba ranarum n. sp., Grassi, 1879.
"Amœba ranarum (?) (mihi)" Grassi, 1882.
"Amöbe" Brass, 1885.
Amœba ranarum (Grassi) Doflein, 1901.
Entamœba ranæ Hartmann, 1907.
Entamœba ranarum (Grassi) Dobell, 1908.

The existence of an amœba in the intestine of the frog was first pointed out by Lieberkühn (1854), whose observations, as far as they went, were very accurate. He was able to distinguish it from leucocytes found in the same place, though it is not certain that the amœba which he saw and figured

was the form which I am about to describe. It is quite possible that he observed the amœboid stage of Chlamydophrys,[1] which I shall describe later. Lieberkühn did not name the organism, and as far as I am aware no name was given to it until 1879, when Grassi proposed the name Amœba ranarum. In the meantime, however, its existence had been recognised by Leuckart and others. Grassi's form is perhaps the same as mine, though this is not certain as no really accurate description of the animal has yet been given. Doflein (14) retained Grassi's name. I presume, moreover, that it is this form to which Hartmann (24) refers as Entamœba ranæ.

It was pointed out by Casagrandi and Barbagallo (54) that the parasitic amœbæ should probably be separated generically from the free-living Amœba. They proposed the new genus Entamœba, therefore, to contain the parasitic forms found in man and in the cockroach. There can be small doubt that this is justifiable. And the proposal was adopted by Schaudinn (43) in his work on the amœbæ in man.

Although the life-history of the amœba in frogs appears to differ considerably from that of other parasitic amœbæ,[2] I think it best at present to place it in the genus Entamœba. Assuming, then, that this organism is the same as that described by Grassi, it follows that its correct name is Entamœba ranarum Grassi.

Lieberkühn stated (37) that the organism occurred frequently in the large intestine of its host, sometimes being present in considerable numbers. He noted that it contained a number of granules, one of which (? the nucleus) was often of specially large size. Ingestion of food and division, though constantly sought, were never observed.

To this account Grassi (21) added the following facts.

[1] This also applies to the amœbæ described in frogs by other investigators—especially Grassi, whose description corresponds much more closely with Chlamydophrys than with Entamœba.

[2] And though E. coli and E. muris are very much alike, they appear to differ very greatly from E. blattæ as regards life-history.

The organism occurs in Rana esculenta captured in Rovellasca, Pavia, and Como. It is invariably present at all seasons and is sometimes very abundant. It was never found in toads. Regarding its structure, he says that an ectoplasm and endoplasm are distinguishable; that there is a round nucleus, with a nucleolus; that the form of the body is very variable, the protoplasm being almost liquid ("scorrevolissimo quasi fosse liquido"). Movement is rapid and effected by the thrusting out of digitiform pseudopodia, which may also be thrust in and out, however, without the animal changing its position. No contractile vacuole was noticed. The dimensions are stated to be as follows: Diameter, when rounded, from 8 μ to 24 μ; when digitiform, up to 30·3 μ in length; diameter of nucleus never greater than 4·4 μ.

Additional statements regarding the life-history have been made by Brass (**2**) and Hartmann (**24**). According to the former, the amœbæ are able to reproduce by division, by formation of swarm-spores and by means of resting spores. According to the latter, an autogamy similar to that of Entamœba coli occurs in Entamœba ranarum, though the observations upon which the statement rests are as yet unpublished.

I will now give my own observations on the animal. They differ in many respects from those of others.

Regarding the host, I can state that Entamœba ranarum occurs in Rana temporaria (Cambridge and Munich), Rana esculenta (Munich), and Bufo vulgaris (Cambridge). I have found it most frequently in Rana temporaria, about 23 per cent. of individuals examined being infected. Occasionally the parasite is present in immense numbers. It is also noticeable that the infection is local, for I often found that nearly all the frogs captured together in certain places were infected, whilst of others taken in a different area not one harboured the parasite.

Whether the animal exercises any injurious effect upon its host or not must remain an open question. Certain it is, however, that sometimes when the parasites are numerous

there are also present many blood-corpuscles and broken-up epithelium cells in the large intestine. These are readily ingested by the amœbæ. But their presence may be due, as Neresheimer (**40**) believes, to the injurious action of the intestinal worms which are always present in greater or less numbers.

All attempts to cultivate Entamœba ranarum on Musgrave and Clegg's medium have failed.

Structure.—This amœba is remarkable for the ease with which its structure at all stages of development can be seen during life. For instance, the nucleus can, with proper illumination, etc., be seen in the living animal just as plainly as in a fixed and stained specimen.

When an ordinary individual is examined in the living state it presents all the features usually seen in any amœba (see fig. 52, Pl. 4). There is no sharply-marked differentiation into ectoplasm and endoplasm; there is no contractile vacuole; there are the usual food bodies present more or less plentifully; but the most distinctive feature is the nucleus. By far the greater part of its chromatin is distributed at the periphery, so that in optical section the nucleus is always seen as a beaded ring (figs. 52, 53). Staining shows that a part of the chromatin is also distributed inside in the form of minute granules of varying size, arranged in a more or less distinct network (fig. 53). There is no caryosome (cf. Grassi). In fixed and stained animals the cytoplasm shows a very distinctly alveolar structure (cf. fig. 53, etc.).

It is, of course, very difficult to give exact measurements of an organism such as this. When more or less rounded the ordinary individuals measure about 20—30 μ in diameter. The nucleus is more easily measured. Its diameter is usually about 6 μ (cf. Grassi).

Very much larger organisms are sometimes to be found (fig. 58). They are often stuffed with food to a most surprising extent, but are nevertheless very active. The largest I have found measured over 60 μ in length when in a very slightly extended condition. In these forms the nucleus

becomes modified (fig. 59). It increases in size, reaching a diameter of 8–9 μ, and nearly all the chromatin passes to the periphery, so that the inside is quite pale in a stained preparation.

I have usually met with these large forms in cultures of the fæces. I believe they are to be regarded as abnormal animals, overgrown from over-feeding. Such hypertrophied organisms seem to be incapable of either dividing or encysting. They have always died when kept under observation.

In addition to the ordinary adult animal and the hypertrophied form, there are also to be found amœbæ of much smaller size (fig. 54). They are very much less common, but from the occurrence of all stages intermediate between them and the adults, I have no doubt that they are really the young forms. In addition to their small size they are characterised by possessing a different type of nucleus: for it is spherical, with a small but very distinct karyosomic granule (fig. 54). The diameter of the nucleus is 3–4 μ.

Although the animals must sometimes divide very rapidly—judging from their great abundance occasionally—it is extraordinarily difficult to find stages in division in preparations. I have never seen division in the living animal, though it is not for want of seeking for it. In preparations also I have encountered but few dividing animals, and these, unfortunately were all in approximately the same condition. Fig. 55 shows an organism with a very distinct dividing nucleus. From the occurrence of a binucleate stage (fig. 56), it is probable that fission of the cytoplasm does not take place till some time after that of the nucleus. It seems that the nuclear division is a kind of very primitive mitosis, similar to that seen in the cysts (see infra), where I have been able to follow it in considerable detail.

Occasionally one encounters forms like that depicted in fig. 57, in which an amitotic division of the nucleus is very strongly suggested. But observation of the living animal shows that such a state has absolutely nothing to do with division. The shape of the nucleus is constantly changing

with the movements of the animal, and with the change of position of the food masses lying in the cytoplasm. The condition figured is brought about by the pressure upon the nucleus as it is being forced into the pseudopodium, which is being thrust out. This may be repeatedly seen in living creatures. The nucleus itself is not really amœboid, but undergoes passive distortion.

Encystment.—I have experienced great difficulty in finding any stages in this animal other than those just described. For a long time I could find no indications of encystment, in spite of trying all the means I could think of to bring it about. When I did discover the cysts, however, I came upon them in immense numbers, so that I was able to follow the process of encysting in great detail. All encysting forms were found in December, January and February (cf. the case of the flagellates), but this is perhaps merely a coincidence.

Before encysting, Entamœba undergoes certain changes in its nucleus. The chromatin at the periphery increases in amount and is then gradually extruded[1] into the cytoplasm, where it lies in irregular masses (fig. 60). These masses gradually increase in size by the chromatin granules running together (figs. 61, 64, etc.) The process continues until quite a large quantity of chromatin is lying free in the cytoplasm. At about the same time the nucleus develops a karyosome at its centre. The karyosome always has the structure (seen in figs. 60, 61, 64) of a little heap of loosely packed granules. Fine filaments connecting it with the periphery can usually be distinguished (cf. fig. 60, etc.) The amœba now slowly rounds itself off, and a large vacuole appears in the cytoplasm (fig. 61) When it has reached this stage the organism secretes a delicate cyst membrane (figs. 63, 64). In the living animal these cysts have a very characteristic and striking appearance, with their large nucleus, refractive chromatin masses, and big vacuole (fig. 63).

[1] I have not been able to watch this in the living animal. The statement is based upon a study of fixed and stained material.

Their size is variable; they measure from ca. 10 μ to 16 μ in diameter—on the average 12–13 μ. The diameter of the nucleus is ca. 6 μ.

The cysts remain in this uninucleate condition for some time—exactly how long I have not been able to determine. Then the nucleus begins to divide. In the living animal it is seen to grow out into a long spindle, which subsequently gives rise to the two daughter-nuclei, but no details can be seen. In stained preparations, however, the whole process of division can be followed out in every stage with extraordinary clearness (see figs. 65–70). The first thing observable is the formation of two little outgrowths at opposite poles of the nucleus (fig. 65). As these gradually draw apart the nucleus assumes the characteristic and remarkable spindle-figure (fig. 66). The karyosome lies at first in the middle of the spindle (fig. 65), but subsequently its component granules dispose themselves on the longitudinally arranged spindle fibres, as shown in fig. 67. At this stage the spindle extends almost from one side of the cyst to the other. Subsequent differentiation into two daughter-nuclei takes place through the rearrangement of the karyosomic granules to form two daughter-karyosomes, and through the subsequent constriction of the middle of the spindle (figs. 68, 69). When this is finally completed two daughter-nuclei, exactly like the original nucleus except that they are smaller, are seen inside the cyst (fig. 70).

In this binucleate condition the cyst remains for but a short time. Then both nuclei divide. They do not divide in quite the same way as the original nucleus. Instead of forming a spindle by outgrowth at opposite poles, as originally happened (fig. 65), they each form a spindle by outgrowth at one pole only (cf. figs. 71, 72, 73).[1] The karyosome therefore lies at

[1] It might be thought that these two nuclei (fig. 71) are not about to form the second spindles, but are just finishing the first division—the drawn-out poles being the points which were connected, as in the right-hand pair of nuclei in fig. 74. Many considerations render such an interpretation highly improbable. In the first place, in the living

one end of the spindle at the beginning of division (fig. 72), the "spindle" itself being really club-shaped. As the spindles draw out the karyosome granules travel along the spindle-fibres (fig. 73, lower spindle), and finally some of them reach the opposite end and give rise to the distal daughter-karyosome (fig. 73, upper spindle). Constriction of the spindle then takes place, as in the case of the first spindle, and finally four nuclei are formed in the cyst (figs. 74, 75). As will be apparent from the figures, the two secondary nuclei do not always divide simultaneously.

The next thing which happens is the removal of the chromatin masses in the cytoplasm. In many cases this is apparently absorbed, for it stains paler and paler, and gets smaller and smaller, and all fully-developed cysts are quite without free chromatin (fig. 77). But I have found a number of cysts which appear to indicate quite clearly that the chromatin is sometimes removed directly by extrusion from the cyst through the membrane (cf. fig. 76). Apparently, therefore, it may be got rid of in either way.

In addition to losing its chromatin masses the cyst now loses its vacuole (figs. 76, 77, 79). After this the cyst becomes slightly thicker and more yellow in colour. It contains the four nuclei (the product of the two nuclear divisions), each of which has a very characteristic appearance—that of a ring with a central karyosome granule (fig. 77). The structure of the nuclei can be seen quite clearly in the living cyst (fig. 79). Each nucleus measures about 3 μ in diameter—that is, half the diameter of the original single nucleus which was present in the cyst (fig. 64).

---

animal the spindles are seen to grow across from one side of the cyst to the other. Then, again, we should expect to find the projections turned towards one another if they were the results of the first division. But actually they are often directed in opposite directions (cf. fig. 71). It should also be noted that the spindles of the second division remain pointed at only one end until quite late in development (cf. both spindles in fig. 73). All the facts are in favour of the interpretation given above.

When the cysts reach this stage they cease to develop. I have never found cysts containing more than four nuclei.

The cysts remain in this condition for many days if left in water. If dried they invariably die. But even in the water a large proportion of cysts always degenerated, gradually breaking up (fig. 78).

Attempts to cause the cysts to develop further in the intestinal juices of frogs have always been negative—as I believe, owing to the abnormal state of the frogs used (cf. pp. 213, 264).

If we compare the nucleus of the smallest kind of amœba found in the frog (fig. 54) with the nuclei in the fully-formed cyst (fig. 77) we cannot fail to be struck by their similarity. They correspond closely in structure and size. It appears to me probable that when the cyst reaches its new host's gut its contents break up into four small amœbæ, which are then set free and grow up into the ordinary form, just as in Entamœba coli the cysts liberate broods of eight (Schaudinn [**43**]).

Perhaps it may also be inferred, from the kind of chromatin reduction which takes place during encystment, that the four nuclei are reduced gamete nuclei, and the small amœbæ liberated from the cysts conjugate with one another. But this is mere hypothesis. The history of the chromatin is, at all events, suggestive.

Now it must be apparent to anyone reading this description and looking at the figures that at certain stages of development there is an extraordinary resemblance to certain stages in the autogamy of E. coli (Schaudinn [**43**]) and E. muris (Wenyon [**87**]). Indeed, had one encountered isolated stages, and had one not been able to follow up every stage of development, one would be strongly inclined to believe that an autogamy occurred also in E. ranarum. (Compare some of the figures in Pl. 4 with those of Wenyon, e. g. the cyst with two spindles [fig. 72], with a similar cyst [fig. 23, Pl. 10] of Wenyon's paper). I do not for a moment suggest that Schaudinn and Wenyon were guilty of misinterpreting

what they saw. I admire greatly the work of both. I merely draw attention to the resemblance.

I have even found stages which might at first sight be thought to show a condition in which the nucleus was being completely analysed into chromidia (fig. 62). From comparison of the "chromidia" with the micro-organisms in the same preparation, and also from what I have seen in the living animal, I have no hesitation in saying that the "chromidia" are really bacteria, and we are here dealing with a case of bacterial invasion, in which the nucleus is attacked as well as the cytoplasm. The animals appear particularly liable to the attacks of bacteria just before forming the cyst membrane.

From what I have already said it will be apparent that I can confirm neither the statements of Brass as regards spore-formation, nor those of Hartmann[1] regarding autogamy in E. ranarum. On the contrary, I have found that the nucleus undergoes a perfectly straightforward series of changes leading up to the formation of a quadrinucleate cyst, which probably serves for the dissemination of the organism.

The nuclear divisions in Entamœba ranarum present some interesting features. Division does not seem to correspond with any of the forms hitherto described. Mitosis was first described in an Amœba by Schaudinn (**78**), and he also gave (**76**) the first accurate description of amitosis in the genus. Similar observations have been made on other forms by other observers since. The nuclear division I have just

[1] After writing the foregoing remarks I received Hartmann's paper on an amœba (Entamœba tetragena Viereck = E. africana Hartmann) found in certain cases of dysentery in man. Hartmann believes that an autogamy occurs, but from his figures I have little doubt that future observations on the living animal will show that a condition almost identical with that seen in E. ranarum—as already described in preceding pages—really prevails. Hartmann's figures bear an extraordinary resemblance to isolated stages in the development of E. ranarum. (See Hartmann, Beih. 5, 'Arch. Schiffs. Tropenhygiene,' xii, 1908.

described appears to be neither truly mitotic nor truly amitotic, but rather of an intermediate type.

The difference between the method of formation of the first spindle and that of the second pair in the cysts is as extraordinary as it is unaccountable. I cannot suggest even the slightest reason for it.

In addition to the parasitic amœba found in the intestines of frogs and toads, one sometimes meets with another amœboid organism, which differs in many respects from that just described. As a result of culture experiments with the contents of the intestine, I am now convinced that this organism represents a phase in the life-history of the shelled rhizopod, Chlamydophrys stercorea Cienkowski, which I will now describe. I may mention also, en passant, that minute Amœbæ belonging to the limax-group also turn up frequently in cultures made from the fæces. But then they are found quite commonly in organic infusions of almost any kind. Still, their presence, and that of leucocytes, offer difficulties to the investigator, and for that reason I mention the fact.

(2) Chlamydophrys stercorea Cienkowski.

Syn.: [? Difflugia enchelys (Ehrbg.) Cienkowski, 1876].
Troglodytes zoster Gabriel, 1876.
Platoum stercoreum (Cienkowski) Bütschli, 1880.
Leydenia gemmipara Schaudinn, 1896.
Chlamydophrys stercorea (Cienkowski) Schaudinn, 1903.

This very interesting rhizopod was first described and named by Cienkowski in 1876 (5). He says it is the same organism that Schneider[1] described under the name Difflugia enchelys Ehrbg., but I think there can be no doubt that it is not. D. enchelys Ehrbg., is really the same as Trinema

[1] 'A. Schneider, 'Muller's Arch. Anat. Physiol.,' 1854, p. 191.

acinus Duj., described by Dujardin, Claparède and Lachmann, F. E. Schulze, and others (see their descriptions and figures). In the very same year that Cienkowski's work appeared a remarkable account of an organism, named Trog-lodytes zoster, was published by Gabriel (**19**). From his description I feel almost convinced that he really observed the same organism. This work is remarkable in that it anticipates the discovery of many of the stages in the life-history of this animal, with which we have since become acquainted through the labours of Schaudinn (**43**). Unfortunately Schaudinn's full description never saw the light, so that for the present our knowledge rests upon his lucid but brief preliminary paper. Many points still require confirmation, therefore; for instance his statement of its identity with Leydenia gemmi-para Schaud., the amœboid organism described by Leyden and Schaudinn (**36**) in ascitic fluid. Taking the results of Cienkowski, Gabriel and Schaudinn together, we appear now to have a fairly perfect knowledge of the life-cycle of Chlamydophrys. Nevertheless, as the work requires confirmation I think my observations may not be superfluous.

According to Bütschli (**3**) Chlamydophrys is a synonym for Platoum F. E. S. But this is really a free-living form, similar to, but not the same as, Chlamydophrys.

As is well known, Chlamydophrys is an animal which lives in the fæces of various animals, the cysts passing along the alimentary tract before they undergo development. It is still unknown whether the forms of Chlamydophrys found in the fæces of different animals represent one species or several. This can be decided only by further research.

I have found the organism in Rana temporaria and Bufo vulgaris, but not frequently. In both the animal appears to be identical.

Schaudinn was the first to observe that the animals might escape from their cysts in the form of an amœba before leaving the intestine of the "host." This happens only occasionally.

The Chlamydophrys amœbæ, which I have found in the

large intestine or in the discharged excrement of frogs, have a very characteristic appearance (see Pl. 5, figs. 81, 82). They are active and show a well-marked ectoplasm and endoplasm (fig. 81). The protoplasm itself often shows a most striking alveolar structure, which is no less marked in the living animal than in a fixed and stained specimen (compare figs. 81 and 82). In the living state also the structure of the nucleus is seen quite as distinctly as in a stained preparation. It is characterised by a large central mass of chromatin, separated by a clear zone from the membrane. Its structure corresponds very well with that figured in "Leydenia" by Schaudinn. It is impossible to mistake this amœba for Entamœba ranarum (compare figs. 81 and 52). A contractile vacuole is sometimes, but not always, to be seen. When present it can often be seen to arise as several small vesicles, which fuse together during the diastole.

Although the amœbæ are sometimes to be found in large numbers I have never succeeded in finding dividing forms. According to Schaudinn they are capable of multiplying both by equal bipartition and by budding.

It is interesting to note that they will live and apparently multiply—though I have never found dividing individuals—in saline albumen solution. On one occasion when I transferred some amœbæ into a culture dish containing the albumen solution, I found that after the lapse of twenty-four hours they had increased considerably in numbers and were very active. Owing to drying of the solution many of the amœbæ subsequently encysted. This ability to live thus is of interest in connection with Schaudinn's statement that "Leydenia" is really the Chlamydophrys amœba.

After creeping about in the fæces for some time the amœbæ come to rest and develop into the typical adult form. They do this by rounding themselves off and developing a shell—a thin, shining, white, porcellaneous structure. It is oval in shape, with the opening at the apex (see fig. 80, Pl. 5). Through the opening, the animal protrudes delicate filose pseudopodia, which serve to catch its food.

A good deal of variation is seen in the size of the animals. The measurements correspond very closely with those given by Gabriel for "Troglodytes." An average large-sized individual measures about 20 μ by 14 μ.

Inside its shell the animal's body is roughly differentiated into two zones—an anterior vacuolate zone, lying immediately behind the shell aperture, and a posterior non-vacuolate zone containing the nucleus. As the food particles reach the interior through the shell opening they appear to be digested entirely in the vacuolate zone. In this zone contractile vesicles are also to be found sometimes. Their number varies, and they are not always present. No distinct partition into two zones—as in Difflugia, Euglypha, etc., occurs.

According to Schaudinn the nucleus is surrounded by a chromidial mass, which subsequently breaks up to form the gamete nuclei—usually eight in number. I have never been able to find a distinct chromidial net, though the protoplasm in the posterior region is often very dense, and contains many darkly-staining granules. Possibly these are the chromidium in its early stages. Later stages, with gamete formation, have never come under my notice.

The nucleus itself is precisely the same as in the free-living amœboid form. Occasionally more than one nucleus is present, as Cienkowski long ago noticed.

When the cultures containing Chlamydophrys were allowed to dry the animals very readily encysted. This happened even before the animal had developed its shell. But the shelled forms also encysted, the protoplasm flowing out of the shell-opening becoming spherical and secreting its cyst wall outside. The shells subsequently broke up. On one occasion I found an animal which had encysted inside its shell (fig. 84), but this is very unusual.

The cysts themselves (fig. 83) vary enormously in size. The smallest are about 6 μ in diameter, the largest 16–17 μ. An average size is 14 μ. Their most striking feature is their thickness, which is often very great in places. They are

very irregular externally and always heavily encrusted with foreign bodies (cf. fig. 86). Their colour is brown or brownish-yellow. They are not unlike those of Copromonas, though usually to be distinguished by their excrescences.

As a rule only one nucleus can be seen in each cyst. Once, however, I found a cyst containing two individuals, each with a nucleus (fig. 85). This is rare.

Schaudinn found that, in order to develop, the cysts had to pass through the alimentary canal of the "host" animal. But this is not the case with the Chlamydophrys from frogs. Perhaps the cysts I observed were only temporary, and not the same as the durable structures which arise after conjugation. At all events, I found that moistening the dried fæces sufficed to cause a number of animals to emerge from their cysts. It appears to be immaterial whether the fæces are moistened with salt-solution, water, or juice from the intestine. In each case the cyst-wall dissolved, and the animal emerged and began life once more as an amœba. (See Pl. 5, figs. 87–90, which show emergence of an amœba from a cyst.) A considerable percentage of cysts never dissolved. A good many showed protoplasmic streaming after the addition of liquid to the fæces, but after a time it ceased and the cysts showed no further signs of life.

At the time when I encountered Chlamydophrys most abundantly I was unfortunately so busily engaged in working at other forms that I was unable to take proper care of the cultures. The result is that what few further observations I was able to make, though interesting and curious, were too uncertain to carry much weight. In consequence I cannot add anything more to the account of the life-history already given by Schaudinn.

I may remark that the "Amœba sp." which Wenyon describes in the mouse, in addition to Entamœba muris, is perhaps also Chlamydophrys, or an allied form.

c. Sporozoa.

In my preliminary note (**12**) I recorded the presence of a new coccidian in the intestine of the frog. I then gave the name Coccidium ranæ to the organism. But having since made a more careful study of the literature, I am reluctantly compelled to relinquish the generic name Coccidium in favour of the apparently more accurate Eimeria. That a long-familiar name like Coccidium should have to be completely abolished is indeed deplorable. I feel convinced, however, that the retention of this name (as Schaudinn and Minchin have retained it) is really unjustifiable. Unless our system is to be thrown into absolute disorder there can be no place for anarchy in zoological nomenclature—whatever one's feelings in the matter may be. The name of this coccidian is therefore—

Eimeria ranæ Dobell.

A few words must first be said to justify the specific distinction given to this animal, as several coccidian parasites have already been recorded in frogs. The history of these is briefly as follows:

Lieberkühn (**37**) was the first to describe "psorosperms" in frogs. He found these in the kidneys only, not in the intestine. The parasite described by him is that now known as Isospora lieberkühni Labbé. In 1870 Eimer (**17**) found in frogs the developmental forms of a coccidian, which he considered was probably the same as that which he observed in the mouse (Eimeria falciformis Eimer[1]). Pachinger (**41**) also found intestinal coccidia—in the duodenum of Rana esculenta. He gave the name Molybdis entzi to them, but gave an insufficient description of their structure. It is thus impossible to know whether the parasites described by Eimer and Pachinger correspond with my form or not.

[1] Called by him "Gregarina" falciformis, however.

Labbé (30) mentions that he found a parasite, like that occurring in newts, in the nuclei of the intestinal epithelium of Rana temporaria. Without giving any further description he bestows the name Karyophagus ranarum n. sp. upon it. But on the very next page (p. 212) he says that he believes that this parasite is identical with Karyophagus salamadræ Steinhaus and Cytophagus salamandræ Steinhaus. And he proposes to call them all Acystis parasitica! Later (31) Labbé retains the name Caryophagus ranarum Labbé for the intestinal coccidian of the frog, but gives the host as Rana esculenta, and gives no further description of it. It is obviously useless to attach much importance to these names, and impossible to identify the animal.

The only careful work which has been done upon the coccidian parasites of frogs is that of Laveran and Mesnil. But none of the forms described by them appear to correspond with my form. These two investigators have worked out the whole of the life cycle of the organism found by Lieberkühn, and have discovered some very interesting details (Laveran et Mesnil [32]). Of special interest is the fact that this parasite, though normally attacking the kidneys, may give rise to a general infection of the host. And in such cases the small intestine may be infected. However, this animal has nothing to do with the form under consideration: it is an Isospora, with disporic oocyst and tetrazoic spores. Laveran and Mesnil described also (33) two more coccidians (from Rana esculenta), giving them the names Coccidium ranarum and Paracoccidium prevoti. The latter differs from all other coccidia in that the sporocyst dissolves in the later stages of development, so that the sporozoites come to lie freely in the oocyst. The former, however, presents many points of resemblance with my parasite. But it differs in several points, the most important being the absence of an oocystic residuum. Quite recently Mesnil (38) has found another coccidium—an Isospora—in the gut of Hyla arborea.

It thus appears to me certain that the parasite under

discussion has not previously been described, and must hence be made a new species.

With regard to the occurrence of Eimeria ranæ, I can record the facts that it was found in Rana temporaria (Cambridge and Munich), in ca. 15 per cent. of all frogs examined,[1] and upon a single occasion in Rana esculenta (Munich).

I have always encountered the stages of the sporogony of this organism in the lower end of the frog's gut—about the posterior half of the small intestine, together with the large intestine. Although I have cut a large number of sections and made repeated examinations of the epithelium of the small intestine and the liver, both in frogs containing spores and in those apparently uninfected, I have never succeeded in finding the slightest trace of the schizogony. I have also examined the kidneys without result, but the distribution of the spores seems to exclude the possibility of these being the seat of schizogony. It appears most probable that schizogony takes place in the small intestine—in the upper part—and is completed before any of the parasites proceed to spore formation. Hence the presence of oocysts in the rectum indicates that schizogony is finished.

Sporogony.—I have been able to follow the whole of the sporogony of this coccidian in the living animal. I have completely failed to obtain stained preparations at any stage, owing to the extraordinary impermeability of the oocysts and spores. Every method has proved unavailing. Even the methods which I have found successful in other cases—dilute acid Delafield's hæmatoxylin, acid alcoholic carmine, etc.—have quite failed in this case. I have left the oocysts, etc. in these stains for over two months without any staining whatsoever taking place. The following is the series of stages to be seen in the living organisms:

The earliest stage found is that shown in Pl. 5, fig. 92. The oocyst is already well developed, and the contents of the cyst are seen to consist of a dense mass of very highly

[1] But it occurs more frequently in the Cambridge frogs.

refractive bodies (reserve material). The cysts are spherical or somewhat ovoid and measure 18–22 μ in diameter. Occasionally—as in fig. 92—a clear area can be seen in the centre, in optical section. This is probably the nucleus.

If such a cyst be kept under observation for some time it is seen gradually to undergo internal changes. The contents slowly become divided up into five masses—at first irregular, but subsequently recognisable as four sporoblasts and an oocystic residuum (fig. 93). This process of segmentation is very slow, and takes from about twelve to twenty hours for completion. No nuclear changes were ever made out.

The changes which now ensue concern the metamorphosis of the sporoblasts into spores. At first the sporoblasts are spherical, with a diameter of about 7·5 μ (fig. 94). In course of time they become oval, however, measuring about 10 μ by 7 μ. They then begin to show a clear area of protoplasm at one spot, quite free from the refractive bodies (fig. 95). The time taken to reach this stage is about another twenty hours or so after the spherical sporoblasts are clearly differentiated.

Subsequently the sporoblasts slowly change into spores. They acquire a membrane, and later begin to have a "pseudo-navicella"-like appearance (fig. 96). Inside the developing spore the refractive bodies heap themselves into a spherical mass, which later represents the sporal residuum. This stage is reached after about another six to seven hours.

From this stage onward development proceeds more slowly. The spore-membrane thickens, acquiring a very evident double contour, with a knob-like eminence at either end. The spores are now markedly "pseudo-navicella"-like in shape (fig. 97). Inside the spore the clear protoplasm is very sharply marked off from the residual mass, which now lies centrally. The clear protoplasm can be seen gradually to become divided in a longitudinal direction into two sporozoites, which lie with their ends curled over one another, tête-bêche (fig. 97). With careful arrangement of the illumination it can be seen that each sporozoite has a nucleus situated towards the middle of its body (fig. 97). They lie

quite motionless inside the spores. When the latter are fully formed the oocyst usually collapses over them (fig. 91), so that they remain loosely encapsuled together. Sometimes the oocyst completely breaks up if kept in a liquid medium, and the spores then become free. They measure ca. 14 μ by 7 μ. Their resemblance to the spores of Monocystis is often very striking in early stages of development. As I have already noted (p. 206), these spores are not uncommon in frogs. Of course, when fully formed the octozoic Monocystis spores cannot possibly be mistaken for the dizoic spores of the Eimeria.

As in all the other forms investigated, I have found great difficulty in causing the contents to emerge. The gastric juice of the frog is quite without action upon the spores. So also are 2 per cent. HCl, 3 per cent. $Na_2 Co_3$, and artificial solutions of trypsin or pepsin. The juices from the small intestine of laboratory frogs is also, as a rule, without effect. On a single occasion, however, when I used the juice from the upper part of the small intestine of a frog killed almost immediately after it was captured, I saw the following events take place: In three or four spores lying near to one another, the sporozoites —after about a quarter of an hour—began to move about inside their spores. The movements increased, and finally the sporozoites were seen in a state of great activity, chasing one another round and round inside their narrow prison, jostling the residual mass. After this had continued for another hour one spore suddenly burst and a sporozoite emerged. But it then, almost at once, ceased to move, and, after swelling up, died and broke into fragments. The other sporozoites all became motionless subsequently, and none of them came out of their spores. All other attempts to get them to emerge have been fruitless.

I think this experiment indicates that the spores are probably dissolved, and the sporozoites emerge, in the upper part of the small intestine of the frog. The reason that experiments are nearly always negative is probably to be sought in the changes which the digestive juices undergo in

frogs kept in the laboratory. The juices seem to become inactive after the frogs have been kept in captivity without receiving their usual food.

Metzner (**67**) has already pointed out that the spores of Eimeria stiedæ are opened by pancreatic and not gastric juice—a condition which probably obtains also in E. ranæ.

In conclusion, I may call attention to the similarity which exists between the sporogonic stages of this coccidium and those of Eimeria salamandræ, Steinh. (see Simond's figures, etc.). The schizogony and fertilisation of this animal are now known, through the work of Steinhaus, Simond and others (cf. **83**, **82**, **81**, **14**).

D. Ciliata.

I am able to add but little to the life-history of the Infusoria which occur in the frog. The two following observations, however, appear to me worth recording.

(1) Encystment of Nyctotherus cordiformis Ehrbg.

As far as I know the cysts of this animal have not been described hitherto: which is not surprising, as they are exceedingly rare. When removed from the frog Nyctotherus nearly always dies.

The cyst is shown in Pl. 3, fig. 51. It is a more or less oval structure, between 80 μ and 90 μ in length (that figured measured 87 μ). Its colour is greenish-yellow, and it shows a very distinct striation, the striæ following the lines in which the cilia were disposed in the free animal. The mouth and gullet can be seen, though somewhat indistinctly. The meganucleus is very distinct, but I have never been able to distinguish a micronucleus with certainty. One or more vacuoles may be present. They continue to pulsate for some time after the cyst has been formed.

I have kept the cysts in water for over a week, but they finally died. They do not seem able to withstand drying.

(2) Culture of Balantidium entozoon Ehrbg.

I have been able to watch division and encystment in this animal on many occasions, but these have been already described by others in more or less detail. The following observations are of interest in relation to the species question —many different vertebrates harbouring a Balantidium resembling B. entozoon.

I have found that it is possible to cultivate this organism in infusions made from the fæces of a variety of different animals (rats, snakes, etc.). The interest lies in the fact that they not only survive, but also remain extraordinarily active and multiply by frequent division. They will continue to do this for days, so that it is thus possible for these parasites to live and increase also as saprophytes.

I have found cysts in these cultures, but whether they were formed there or originally introduced I cannot say.

It may be added that Balantidium can also exist in certain organic infusions for a considerable time.[1]

In conclusion, I gladly take this opportunity of offering my warmest thanks to Professor Richard Hertwig for his kindness to me whilst working in the Zoological Institute in Munich. I wish also to thank Dr. Richard Goldschmidt for the friendly interest he took in my work whilst there, and for his readily offered advice. But my greatest debt of gratitude is owing to Professor Adam Sedgwick. What measure of success I have achieved is due largely to his inspiration and encouragement—without which I should never have undertaken these researches. I desire, therefore, to thank him most sincerely, as some slight acknowledgment of my indebtedness.

[1] Walker has quite recently published an account ('Journ. Med. Research,' xviii, 1908) of successful cultivation experiments made by him on the flagellates and ciliates in frogs. He states that he has been able to cultivate Nyctotherus, Trichomonas, and "Cercomonas" (? Octomitus) on agar media. Neither he nor Strong ('Bull. Gov. Lab.,' Manila, 1904), however, has succeeded in cultivating Balantidium coli. For my own part, I have not been able to cultivate the flagellates of frogs on Musgrave and Clegg's medium for more than a few days.

Literature.

A.

[This list contains only those memoirs which are directly concerned with the organisms described in the paper. For other references see list B. infra].

1. Blochmann, F.—"Bemerkungen über einige Flagellaten," 'Zeitschr. wiss. Zool.,' xl, 1884, p. 42.
2. Brass.—"Die thierischen Parasiten des Menschen," Cassel, 1885 [quoted from Leuckart, q.v.].
3. Bütschli, O.—"Protozoa" in Bronn's 'Tierreich,' 1880–87.
4. Burnett, W. I.—"The Organic Relations of Some of the Infusoria, including Investigations Concerning the Structure and Nature of the genus Bodo (Ehr.)," 'Boston Journ. Nat. Hist.,' vi, 1850–57, p. 319.
5. Cienkowski, L.—"Über einige Rhizopoden und verwandte Organismen," 'Arch. mikr. Anat.,' xii, 1876, p. 15.
6. Dale, H. H.—"Galvanotaxis and Chemotaxis of Ciliate Infusoria," Part I, 'Journ. Physiol.,' xxvi, 1901, p. 291.
7. Diesing, C. M.—"Systema Helminthum," Vienna, 1850.
8. ——— "Revision der Prothelminthen. Abteilung: Mastigophoren." 'Sitzb. math.-nat. Cl. kaiserl. Akad. Wiss. Wien,' lii, 1865, p. 287.
9. Dobell, C. C.—"Physiological Degeneration in Opalina," 'Quart. Journ. Micr. Sci.,' 51, 1907, p. 633.
10. ——— "The Structure and Life-History of Copromonas subtilis nov. gen. nov. spec.," 'Quart. Journ. Micr. Sci.,' 52, 1908, p. 75.
11. ——— "Notes on some Parasitic Protists," 'Quart. Journ. Micr. Sci.,' 52, 1908, p. 121.
12. ——— 'On the Intestinal Protozoan Parasites of Frogs and Toads" (prelim. com.), 'Proc. Cambridge Phil. Soc.,' xiv (iv), 1908, p. 428.
13. ——— "Some Remarks on the 'Autogamy' of Bodo lacertæ, Grassi," 'Biol. Ctrbl.,' xxviii, 1908, p. 548.
14. Doflein, F.—'Die Protozoen als Parasiten und Krankheitserreger.' Jena, 1901.
15. Dujardin, F.—'Histoire Naturelle des Zoophytes: Infusoires.' Paris, 1841.
16. Ehrenberg, C. G.—'Die Infusionsthierchen als vollkommene Organismen.' Leipzig, 1838.

17. Eimer, T.—'Die ei- und kugelförmigen sogenannten Psorospermien der Wirbelthiere.' Würzburg, 1870.
18. Foà, A.—"Ricerche intorno a due specie di flagellati parassiti," 'Atti R. Accad. Lincei,' (5) xiii, 1904, p. 121.
19. Gabriel, B.—"Untersuchungen über Morphologie, Zeugung und Entwickelung der Protozoen," 'Morph. Jahrb.,' i, 1876, p. 535.
20. Grassi, B.—"Dei protozoi parassiti e specialmente quelli che sono nell'uomo," 'Gaz. Med. Ital.-Lombard.,' xlv, 1879.
21. ——— "Intorno ad alcuni Protisti endoparassitici, etc.," 'Atti Soc. Ital. Sci. Nat.,' xxiv, 1882.
22. ——— "Sur quelques protistes endoparasites," 'Arch. Ital. Biol.,' ii, 1882, p. 402; and iii, 1883, p. 23.
23. ——— "Morfologia e sistematica di alcuni parassiti," 'Atti R. Accad. Lincei,' iv (1), 1888, p. 5.
24. Hartmann, M.—"Das System der Protozoen," 'Arch. Protistenk.,' x, 1907, p. 139.
25. ——— und Prowazek, S. v.—"Blepharoplast, Caryosom und Centrosom. Ein Beitrag. zur Lehre von der Doppelkernigkeit der Zelle," 'Arch. Protistenk.,' x, 1907, p. 306.
26. Kent, W. Saville.—'A Manual of the Infusoria.' London, 1880–82.
27. Klebs, G.—"Flagellatenstudien," 'Zeitschr. wiss. Zool., lv, 1892, pp. 265 and 353.
28. Kunstler, J.—"Sur cinq Protozoaires parasites nouveaux," 'CR. Acad. Sci. Paris,' xcv, 1882, p. 347.
29. ——— et Gineste, C.—"Giardia alata (nov. spec.)," 'CR. Acad. Sci. Paris,' cxliv, 1907, p. 441.
30. Labbé, A.—"Recherches . . . sur les parasites endoglobulaires du sang des Vertébrés," 'Arch. Zool. Exp.' (3), ii, 1894, p. 55.
31. ——— "Sporozoa," in 'Das Tierreich.' Berlin, 1899.
32. Laveran, A., et Mesnil, F.—"Sur la coccidie trouvée dans le rein de la Rana esculenta et sur l'infection générale qu'elle produit," 'CR. Acad. Sci. Paris,' cxxxv, 1902, p. 82.
33. ——— ——— "Sur deux coccidies intestinales de la Rana esculenta," 'CR. Soc. Biol.,' liv, 1902, p. 857.
34. Leidy, J.—"A Synopsis of Entozoa and some of their Ecto-congeners," 'Proc. Acad. Nat. Sci. Philadelphia,' viii, 1856.
35. Leuckart, R.—'Die Parasiten des Menschen,' 2 Aufl., 1879–86.
36. Leyden, E., und Schaudinn, F.—"Leydenia gemmipara Schaudinn, ein neuer . . . Rhizopode," 'Sitzb. Akad. Wiss. Berlin,' xxxix, 1896, p. 951.
37. Lieberkühn, N.—"Über die Psorospermien," 'Müller's Arch. Anat. Physiol.,' 1854, p. 1.
38. Mesnil, F.—"Sur un coccidie de la Rainette," 'CR. assoc. franç. avanc. sci. Congrès de Reims,' 1907.

39. Moroff, T.—"Beitrag zur Kenntniss einiger Flagellaten," 'Arch. Protistenk.,' iii, 1903, p. 69.
40. Neresheimer, E.—"Die Fortpflanzung der Opalinen," 'Arch. Protistenk.,' Suppl. 1, 1907, p. 1.
41. Pachinger, A.—"Mittheilung über Sporozoen," 'Zool. Anz.,' ix, 1886, p. 471.
42. Perty, M.—'Zur Kenntniss kleinster Lebensformen, usw.' Bern, 1852.
43. Schaudinn, F.—"Untersuchungen über die Fortpflanzung einiger Rhizopoden (Vorl. Mitteil.)," 'Arb. kaiserl. Gesundheitsamte,' xix, 1903, p. 547.
44. Seligo, A.—"Untersuchungen über Flagellaten," Inaugural Dissertation, Univ. Breslau, 1885.
45. Senn, G.—"Flagellaten" in Engler und Prantl's 'Nat. Pflanzenfamilien,' 1900.
46. Stein, F.—'Der Organismus der Infusionthiere; III Abtheilung: Flagellaten.' Leipzig, 1878.
47. Stiles, C. W.—"The Type-species of Certain Genera of Parasitic Flagellates, particularly Grassi's Genera of 1879 and 1881," 'Zool. Anz.,' xxv, 1902, p. 689.

B.

[Papers not dealing directly with the organisms in frogs and toads.]

48. Belajeff, W.—"Über den Bau und die Entwicklung der Antherozoiden, 1. Characeen," 'Sitzb. Warsch. naturf. Ges.,' 1892.
49. ——— "Über Bau und Entwicklung der Spermatozoiden der Pflanzen," 'Flora,' liv, 1894.
50. ——— A series of papers on Antherozoids, etc., in 'Ber. deutsch. bot. Ges.,' 1897–1899.
51. Bohne, A., und Prowazek, S. v.—"Zur Frage der Flagellatendysenterie," 'Arch. Protistenk.,' xii, 1908.
52. Bradford, J. R., and Plimmer, H. G.—"The Trypanosoma brucii, the Organism found in Nagana, or Tsetse-fly Disease," 'Quart. Journ. Micr. Sci.,' 45, 1902.
53. Bütschli, O.—"Beiträge zur Kenntniss der Flagellaten und einiger verwandten Organismen," 'Zeitschr. wiss. Zool.,' xxx, 1878.
54. Casagrandi, O., e Barbagallo, P.—"Ricerche biologiche e cliniche sull' Amœba coli (Lösch), 2ª nota prelim.," 'Boll. Accad. Gioenia Sci. Nat. Catania,' xli, 1895.
55. Certes, A.—"Note sur les parasites et les commensaux de l'huître," 'Bull. Soc. Zool. France," vii, 1882.

56. Dobell, C. C.—"Trichomastix serpentis, n.sp.," 'Quart. Journ. Micr. Sci.,' 51, 1907.

57. França, C., et Athias, M.—"Recherches sur les Trypanosomes des Amphibiens; II, Le Trypanosoma rotatorium de Hyla arborea," 'Arch. Inst. Bact. Camara Pestana,' i, 1907.

58. Grassi, B., e Foà, A.—"Ricerche sulla riproduzione dei Flagellati; I, Processo di divisione delle Joenie," 'R.C. Accad. Lincei Roma,' xiii (5), 1904.

59. Gross, J.—"Die Spermatogenese von Pyrrhocoris apterus L.," 'Zool. Jahrb. (Anat.),' xxiii, 1906.

60. Ikeno, S.—"Untersuchungen über die Entwickelung der Geschlechtsorgane usw. bei Cycas," 'Jahrb. wiss. Bot.,' xxxii, 1898.

61. Ischikawa, C.—"Further observations on the Nuclear Division of Noctiluca," 'Journ. Sci. Coll. Tokyo,' xii, 1899.

62. Kunstler, J.—"Recherches sur les Infusoires Parasites; sur quinze Protozoaires nouveaux," 'CR. Acad. Sci., Paris,' xcvii, 1883.

63. ——— "Observations sur le Trichomonas intestinalis," 'Bull. Sci. France Belgique,' xxxi, 1898.

64. Laveran, A., et Mesnil, F.—"Sur la nature centrosomique du corpuscule chromatique postérieur des Trypanosomes," 'CR. Soc. Biol.' (Mars), 1901.

65. ——— ——— "Sur la morphologie et la systématique des Flagellés à membrane ondulante (genres Trypanosoma Gruby, et Trichomonas Donné)," 'CR. Acad. Sci. Paris,' cxxxiii, 1901.

66. Metzner, R.—"Untersuchungen an Megastoma entericum Grassi, aus dem Kaninchendarm," 'Zeitschr. wiss. Zool.,' lxx, 1901.

67. ——— "Untersuchungen an Coccidium cuniculi," 'Arch. Protistenk.,' ii, 1903.

68. Minchin, E. A.—"Investigations on the Development of Trypanosomes in Tsetse-flies and other Diptera," 'Quart. Journ. Micr. Sci.,' 52, 1908.

69. Moore, J. E. S.—"On the Structural Changes in the Reproductive Cells during the Spermatogenesis of Elasmobranchs," 'Quart. Journ. Micr. Sci.,' 38, 1895.

70. ——— and Breinl, A.—"The Cytology of the Trypanosomes," 'Ann. trop. med. parasitol,' i, 1907.

71. Perroncito, E.—"Über die Art der Verbreitung des Cercomonas intestinalis," 'CB. Bakt. Parasitenk.,' iv, 1888.

72. Plenge, H.—"Über die Verbindung zwischen Geissel und Kern, usw.," 'Verhandl. nat.-med. Vereins Heidelberg,' vi, 1899.

73. Prowazek, S. v.—"Untersuchungen über einige parasitische Flagellaten," 'Arb. kaiserl. Gesundheitsamte,' xxi, 1904.

74. Prowazek, S. v.—"Bemerkungen zu dem Geschlechtsproblem bei den Protozoen," 'Zool. Anz.,' xxxii, 1908.
75. Rabinowitsch, L., und Kempner, W.—"Beitrag zur Kenntnis der Blutparasiten, speciell der Rattentrypanosomen," 'Zeitschr. f. Hygiene,' xxx, 1899.
76. Schaudinn, F.—"Über Kerntheilung mit nachfolgender Körpertheilung bei Amœba crystalligera Gruber," 'SB. Akad. Wiss. Berlin,' xxxviii, 1894.
77. ——— "Camptonema nutans, nov. gen. nov. spec., ein neuer mariner Rhizopode," 'SB. Akad. Wiss. Berlin,' lii, 1894.
78. ——— "Über die Theilung von Amœba binucleata Gruber," 'SB. Ges. naturf. Freunde Berlin,' vi, 1895.
79. ——— "Generations- und Wirtswechsel bei Trypanosoma und Spirochæte," 'Arb. kaiserl. Gesundheitsamte,' xx, 1904.
80. Shaw, W. R.—"Über die Blepharoplasten bei Onoclea und Marsilia," 'Ber. deutsch bot. Ges.,' xvi, 1898.
81. Siedlecki, M.—"Reproduction sexuée et début de la sporulation chez la coccidie des tritons (C. proprium Schn.)," 'CR. Soc. Biol.,' [10] v (1), 1898.
82. Simond, P. L.—"L'évolution des Sporozoaires du genre Coccidium," 'Ann. Inst. Pasteur,' xi, 1897.
83. Steinhaus, J.—"Karyophagus Salamandræ, eine in den Darmepithelzellkernen parasitisch lebende Coccidie," 'Virchow's Arch. path. Anat. Physiol.,' cxv, 1889.
84. Ucke, A.—"Trichomonaden und Megastomen in Menschendarm," 'CB. Bakt. Parasitenk. (1 Abt. Orig.),' xlv, 1907.
85. Wasielewski, V., und Senn, G.—"Beiträge zur Kenntniss der Flagellaten des Rattenblutes," 'Zeitschr. f. Hygiene,' xxxiii, 1900.
86. Webber, H. J.—"Development of the antherozoids of Zamia, etc.," 'Bot. Gazette," xxiii and xxiv, 1897.
87. Wenyon, C. M.—"Observations on the Protozoa in the intestine of mice," 'Arch. Prostistenk.' Suppl. i, 1907.

Zoological Laboratory, Cambridge;
July, 1908.

EXPLANATION OF PLATES 2–5,

Illustrating Mr. C. Clifford Dobell's paper on "Researches on the Intestinal Protozoa of Frogs and Toads."

PLATE 2.

[Figs. 11 and 12 are drawn from living animals. The remainder are from permanent preparations. Fixation: hot sublimate-alcohol (Schaudinn). Stain: Heidenhain's iron-hæmatoxylin. Figs. 1, 2, 5, 7, 8, 9 and 21 counterstained with Bordeaux red. All drawings made under Leitz $\frac{1}{12}$ in. oil-immersion with ocular No. 5.]

Figs. 1–15.—Trichomastix batrachorum.

Fig. 1.—Ordinary vegetative individual, showing general structure.

Fig. 2.—Form with thick axostyle. The attachment of the blepharoplast to the bent axostyle is very clearly seen.

Fig. 3.—Form with very slender axostyle.

Figs. 4–12.—Stages in division.

Fig. 4.—First stage in division. The axostyle has disappeared, there is no nuclear membrane, and the blepharoplast is beginning to divide.

Fig. 5.—The blepharoplast is now elongated, forming a rod with two flagella at either end.

Fig. 6.—The chromatin is arranged in a spindle-shaped mass of small granules, and new flagella have made their appearance. (The young flagella are by no means always so well developed as in this instance.)

Fig. 7.—The chromatin is now arranged in large lumps, and the outgrowth of new flagella is very clearly seen.

Fig. 8.—At this stage the chromatin has travelled in two irregular masses towards the blepharoplasts.

Fig. 9.—A similar stage to the preceding. The rod connecting the daughter-blepharoplasts is very distinctly seen.

Fig. 10.—The large lumps of chromatin have broken up to form the new nuclei of the daughter-monads. A thick rod lies between the two nuclei.

Fig. 11.—A somewhat later stage.

Fig. 12.—The same creature a few seconds later. The protoplasm, after welling in and out rapidly several times, has suddenly been constricted, completely severing the two daughter-monads. Each monad has an axostyle which is half of the rod-like structure which connected the blepharoplasts.

Fig. 13.—Trichomastix which has developed a karyosome in its nucleus and is preparing to encyst.

Fig. 14.—Newly-encysted animal. The flagella have gone and the axostyle is degenerating.

Fig. 15.—Permanent cyst. The axostyle has quite disappeared and the nucleus has taken on its characteristic elongate form, with the blepharoplast lying upon it.

Figs. 16–28.—Trichomonas batrachorum.

Fig. 16.—Ordinary individual, large specimen.

Figs. 17–24.—Stages in division.

Fig. 17.—First stage in division. The axostyle has gone, and the edge of the undulating membrane has split.

Figs. 18, 19, 20, 22, 23, 24.—Various stages in division, corresponding with those shown in Trichomastix. (Cf. figs. 4–10.) The membrane is seen in various stages.

Fig. 21.—In this organism a very distinct spindle figure is seen during the division of the nucleus. Note also the diplosomic blepharoplasts and undulating membranes.

Fig. 25.—Trichomonas about to encyst. Nucleus with karyosome.

Figs. 26, 27.—Two successive stages in encystment. Resorption of axostyle, undulating membrane, etc.

Fig. 28.—Permanent cyst, with elongate nucleus and no axostyle or locomotory organellæ.

PLATE 3.

[Figs. 31, 39, 42, 43, 44, 45, 47, 48 and 51 are drawn from living specimens under Zeiss 2·5 mm. apochromatic water-immersion, comp. oc. 12. Remainder from permanent preparations: figs. 46, 49 and 50 fixed absolute alcohol, stained Giemsa; the others sublimate-alcohol and Heidenhain's iron-hæmatoxylin. Drawn, unless otherwise stated, under Leitz 2 mm. oil-immersion (apochrom.) with comp. oc. 12.]

Figs. 29–41.—Octomitus dujardini.

Fig. 29.—Ordinary individual to show nuclear apparatus, etc.

Fig. 30.—Individual with more consolidated nucleus, crossed axostyles, etc.

Fig. 31.—Living animal, moving slowly—axostyles parallel.

Figs. 32–35.—Various small forms, showing different forms of nucleus, etc. (Zeiss 1·5 mm. apo. oil-imm., comp. oc. 12.)

Figs. 36, 37.—Degenerate forms.

Fig. 38.—Encysted Octomitus.

Fig. 39.—Individual moving about rapidly inside cyst. Drawn just before emerging.

Figs. 40, 41.—Probably represent stages in division.

Fig. 42.—Bodo sp. form fæces of Bufo vulgaris.

Fig. 43.—Another individual, amœboid at hind end.

Fig. 44.—Extremely minute Bodo individual.

Fig. 45.—Cyst containing four organisms—probably Bodos.

Fig. 46.—A six-flagellate organism from fæces of Bufo. (Zeiss 3 mm. apo. oil-imm., comp. oc. 12.)

Fig. 47.—Minute uniflagellate monads from fæces of toad.

Fig. 48.—Two three-flagellate monads, also from fæces of toad.

Figs. 49, 50.—Monocercomonas bufonis—wide and narrow forms. (Zeiss 2 mm. apo. oil-imm., comp. oc. 12.)

Fig. 51.—Cyst of Nyctotherus cordiformis. (The pale, sausage-shaped structure is the nucleus; the smaller, light area, above to the left, is a vacuole. Below this is to be seen the rather faint outline of the mouth and gullet.)

PLATE 4.

[All drawings, unless otherwise stated, are made from permanent preparations, fixed with sublimate-alcohol, and stained with Delafield's hæmatoxylin. Drawings made (unless stated to the contrary) under Zeiss 3 mm. apochromatic homog. oil-immersion (1·40), comp. oc. 12.]

Figs. 52–79.—Entamœba ranarum.

Fig. 52.—Large vegetative individual. Living animal. The nucleus is seen as a very distinct ring-like (in optical section) structure, with a beaded appearance, lying near the centre, surrounded by food masses. (2·5 mm. apo. water-imm. [Zeiss] × c. oc. 12.)

Fig. 53.—Ordinary individual. The typical appearance of the nucleus in a stained specimen is well seen.

Fig. 54.—Smallest kind of amœba found, with characteristic nucleus containing a small karyosome. (Formalin 40 per cent., Heidenhain iron-hæmatox.)

Fig. 55.—Individual with nucleus in process of dividing. (Leitz $\frac{1}{12}$ in. oil-imm. × oc. 5.)

Fig. 56.—Amœba with two nuclei—presumably in a stage just before fission of cytoplasm. (Heidenhain Fe-hæmatox. Leitz $\frac{1}{12}$ in. × oc. 5.)

Fig. 57.—Individual with distorted nucleus. The distortion is brought about by the nucleus being forced into a pseudopodium. The condition suggests—falsely—an amitotic division. (Delafield and eosin. Leitz $\frac{1}{12}$ in. × oc. 5.)

Fig. 58.—Large, actively feeding amœba, with modified nucleus. (Hypertrophied; drawn on smaller scale than other figures. Heidenhain and eosin, Zeiss 2 mm. apo. oil-imm., comp. oc. 6.)

Fig. 59.—Nucleus of same individual more highly magnified (comp. oc. 12.)

Fig. 60.—Amœba about to encyst. Note formation of karyosome and protrusion of chromatin into the cytoplasm (on left of nucleus).

Fig. 61.—Encysting amœba. The karyosome is now very well formed, the chromatin masses are very conspicuous in the cytoplasm, and the vacuole has made its appearance. No cyst membrane is as yet to be seen.

Fig. 62.—An amœba, at a similar stage, which has been invaded and killed by bacteria. These have filled the cytoplasm and attacked the nucleus, thus giving rise to an appearance which suggests a resolution of the nucleus into chromidia. (Heidenhain.)

Fig. 63.—A uninucleate cyst, living animal. Nucleus, chromatin masses, and vacuole well seen. (Leitz 2 mm. oil-imm. apo., comp. oc. 12.)

Fig. 64.—A similar cyst, stained.

Fig. 65.—Nucleus beginning to divide.

Figs. 66, 67.—Two succeeding stages of the spindle figure of the first nuclear division. (Fig. 67, Heidenhain and eosin.)

Fig. 68.—Later stage, in which the spindle is being constricted into two.

Fig. 69.—Still later. The two daughter-nuclei are now clearly differentiated, but not yet separated, owing to a part of the spindle persisting between them. (Heidenhain and eosin.)

Fig. 70.—Cyst containing two nuclei, formed by the division of the original nucleus. (Heidenhain and eosin.)

Fig. 71.—The two nuclei beginning to form the spindles of the second nuclear division. Note the way in which the spindle is being formed by extension of only one pole—not by prolongation between two opposite poles (cf. fig. 65). (Heidenhain and eosin.)

Fig. 72.—Cyst with two fully-formed nuclear spindles.

Fig. 73.—Later stage. The upper spindle is further advanced than the lower.

Fig. 74.—Final stage in second nuclear division.

Fig. 75.—Cyst with four nuclei, after second nuclear division. Compare nuclei—as regards size and structure—with those in figs. 64 and 70. (Heidenhain and eosin.)

Fig. 76.—Extrusion of chromatin masses. The vacuole has now disappeared. (Heidenhain.)

Fig. 77.—Cyst after extrusion of chromatin and collapse of vacuole. The membrane is thicker and yellowish. Four very distinctly outlined nuclei are seen. Compare their structure with that of nucleus in fig. 54.

Fig. 78.—Degenerating cyst, with four nuclei.

Fig. 79.—Living 4-nucleate cyst—same stage as fig. 77. (Leitz $\frac{1}{12}$ in. oil imm. × oc. 5.)

PLATE 5.

[Figures, unless otherwise stated, drawn from living animals, under Zeiss 2·5 mm. apo. water-immersion, comp. oc. 12.)

Figs. 80–90.—Chlamydophrys stercorea.

Fig. 80.—Shelled Chlamydophrys, in fæces of toad. (Formalin 40 per cent. Heidenhain iron-hæmatox.)

Fig. 81.—Amœba stage of Chlamydophrys, from large intestine of toad.

Fig. 82.—Similar organism. (Sublimate-alcohol, Heidenhain).

Fig. 83.—Cyst—encrusted, and with a single nucleus. (Formalin 40 per cent. Heidenhain.)

Fig. 84.—An animal which has encysted inside its shell.

Fig. 85.—Cyst containing two individuals. (Sublimate-alcohol. Heidenhain.)

Fig. 86.—Cyst, more highly magnified. (Comp. oc. 18). Note the peculiar knobs or excrescences on the outside, and the thick encrustment of bacteria, etc.

Figs. 87–90.—Four stages in the development of an amœboid Chlamydophrys from its cyst, after moistening the dried-up fæces with water. (Comp. oc. 6.)

Fig. 91–97.—Eimeria ranæ.

Fig. 91.—An oocyst containing four spores and a residuum.

Fig. 92.—An oocyst (undeveloped) from small intestine of frog.

Fig. 93.—The same oocyst twenty hours later. It contains now four sporoblasts and a residuum.

Fig. 94.—A single sporoblast, more highly enlarged.

Fig 95.—The same, twenty-one hours later.

Fig. 96.—The same—now becoming a spore—seven hours later.

Fig. 97.—The same—now a fully formed spore—thirty-six hours later. Two sporozoites and a sporal residuum are seen inside the "pseudo-navicella"-like spore.